The

American Midland Naturalist

Founded by J. A. Nieuwland, C.S.C.

John D. Mizelle, *Editor*

UNIVERSITY OF MICHIGAN
MAR 10 1955
SCIENCE LIBRARY

•

CONTENTS

The Pterobryaceae of the Southern United States, Mexico, Central America, and the West Indies ..... Charles B. Arzeni 1
Liverworts of Georgia—An Annotated List ..... Don L. Jacobs 68
Toxic Fresh-water Algae ..... William Marcus Ingram and G. W. Prescott 75
Cytotaxonomical Studies on the Northern Bedstraw ..... Askell and Doris Love 88
An Electron Microscope Study of Protozoan Flagella ..... Harley P. Brown and Avalee Cox 106
A Catalogue of the Protozoa and Helminths of North American Rodents. I. Protozoa and Acanthocephala ..... David J. Doran 118
Studies on Monogenetic Trematodes. XVI. Rhamnocercinae, a New Subfamily of Dactylogyridae ..... L. H. Monaco, R. A. Wood and J. D. Mizelle 129
New Strigeids from Minnesota Birds and Mammals ..... Asa C. Chandler 133
A Comparative Study of Detergent Effects on Ascarid Development ..... B. J. Jaskoski 142
Statistical Methods and Techniques for the Measurement of Average Index Ratios of Ostracod Carapaces ..... Erhard M. Winkler 149
The Specific Validity of Epischura massachusettsensis Pearse (Copepoda: Calanoida) ..... Arthur G. Humes 154
Entocythere occidentalis sp. nov., a Cytherid Ostracod Commensal on Western Species of Pacifastacus ..... Eugene N. Kozloff and Donald C. Whitman 159
Nevada Hydrophilidae (Coleoptera) ..... Ira La Rivers 164
Ecology of Simuliid Vectors of Onchocerciasis in Guatemala ..... Herbert T. Dalmat 175
A New Species of Box-Mite from Colorado (Oribatei: Phthiracaridae) ..... Tyler A. Woolley 197
Status and Synonymy of the American Percid Fish Hadropterus scierus ..... Carl L. Hubbs and John D. Black 201
A New Texas Subspecies, apristis, of the Darter Hadropterus scierus, with a Discussion of Variation Within the Species ..... Clark Hubbs 211
Notes on the Food of Three Species of Alberta Amphibians ..... J. E. Moore and E. H. Strickland 221
Observations on the Life Cycles of Painted Turtles (Genus Chrysemys) ..... Fred R. Cagle 225
Notes on the Shorttail Shrew (Blarina brevicauda kirtlandi) in Northern Lower Michigan ..... William O. Pruitt, Jr. 236
The Young of the Woodland Jumping Mouse, Napaeozapus insignis insignis (Miller) ..... James N. Layne and W. J. Hamilton, Jr. 242
Hibernation in the American Black Bear ..... Arthur Svihla and Howard S. Bowman 248

Notes and Discussion
Techniques for Rearing Culex Mosquitoes ..... James B. Kitzmiller and Don W. Micks 253
A Record of the Blind Isopod, Asellus tridentatus, from an Open Stream Habitat ..... Ralph W. Dexter 256

Vol. 52 July, 1954 No. 1

EDITORIAL STAFF

JOHN D. MIZELLE ..... *Zoology*
Editor, University of Notre Dame, Notre Dame, Ind.

B. D. BURKS ..... *Entomology*
U. S. National Museum, Washington, D. C.

ALBERT L. DELISLE ..... *Plant Morphology*
University of Notre Dame, Notre Dame, Ind.

CARROLL LANE FENTON ..... *Invertebrate Paleontology*
404 Livingston Ave., New Brunswick, N. J.

JOHN HOBART HOSKINS ..... *Paleobotany*
University of Cincinnati, Cincinnati, Ohio

GEORGE NEVILLE JONES ..... *Plant Taxonomy*
University of Illinois, Urbana, Ill.

REMINGTON KELLOGG ..... *Mammalogy*
U. S. National Museum, Washington, D. C.

JEAN MYRON LINSDALE ..... *Ornithology*
Hastings Reservation, Robles del Rio, Calif.

GEORGE WILLARD MARTIN ..... *Mycology*
State University of Iowa, Iowa City, Iowa

HUGH M. RAUP ..... *Plant Ecology*
Harvard Forest, Harvard University, Petersham, Mass.

KARL PATTERSON SCHMIDT ..... *Ichthyology and Herpetology*
Chicago Natural History Museum, Chicago, Ill.

NOTE: THE AMERICAN MIDLAND NATURALIST, published by the University of Notre Dame is primarily, though not exclusively, devoted to the Natural History of the Middle West. A wide selection of papers on botany, paleontology and zoology is published quarterly. Each issue contains 256 pages, two of which comprise a volume.

Subscription rate per year $6.00 in the U. S., Mexico and South American countries; $7.00 elsewhere.

Authors are invited to submit carefully prepared manuscripts and requested to limit tables and illustrations as much as possible. Authors are requested to defray illustration costs beyond two full-page (4x6) plates.

The following numbers are out of print: Vol. 1 (1, 4, 5, 8-12); Vol 2 (1-3, 8-9); Vols. 3, 4 (all numbers; Vol. 5 (1, 6-8); Vol. 6 (1, 5, 7-12); Vol. 7 (6); Vol. 8 (2); Vol. 9 (2); Vol. 12 (12); Vol. 14 (1, 5-6); Vol. 15 (4); Vol. 16 (2, 4); Vol. 17 (1); Vol. 18 (1); Vol. 21 (all numbers); Vol. 22 (1); Vol. 23 (all numbers); Vol. 24 (3); Vol. 25 (1, 2); Vol. 26 (2, 3); Vol. 27 (1); Vol. 36 (1); Vol. 37 (2, 3); Vol. 38 (1); Volume groups 1-12, 13-18, 19-49, and 50 contain 12, 6, 3 and 2 issues respectively. Available issues of Vols. 1-6, 50 cents; Vol. 7, 35 cents; Vols. 8-12, 50 cents; Vols. 13-14, 60 cents; Vol. 15, 75 cents; Vols. 16-18, $1.00; Vols. 19-48, 50. $4.00 a volume, single issues $1.75. Vol. 49 Nos. 1, 3, $1.75 each; Vol. 49 No. 2, $4.00.

Inquiries concerning exchanges for specimens, journals, special volumes or duplicate books should be addressed to *The American Midland Naturalist*, Notre Dame, Indiana, where subscriptions are also received. Offers should accompany requests for exchange.

Abbreviated citation: *Amer. Midl. Nat.*

The American Midland Naturalist is indexed in the INTERNATIONAL INDEX.

Entered as second-class matter at Notre Dame, Indiana. Acceptance for mailing at special rate of postage provided for in section 1103; Act of October 3, 1917, authorized on July 3, 1918.

The American Midland Naturalist

Published Quarterly by The University of Notre Dame, Notre Dame, Indiana

VOL. 52 JULY, 1954 NO. 1

The Pterobryaceae of the Southern United States, Mexico, Central America, and the West Indies

Charles B. Arzeni

Firestone Plantations Company, Liberia, West Africa

The study of the mosses of the new world tropics has been under way for a period of over two hundred years, yet with the exception of the Hepaticae, there is probably no other group of plants in that region in which the taxonomy is in a greater state of confusion. Verdoorn (1950) gives a timely account of the conditions in which this division of cryptogamic botany is found, especially in the neotropics. He stated that after collecting in such a region for a period of only several hours, "about one-fourth of the bryophytes we find, we can identify more or less; several others can be identified by the few existing specialists, but of the balance we do not know if they have a name, no name, or perhaps (which is more likely) three or four names." The almost complete absence of monographs and critical revisions of the Musci in these areas makes it impossible, even for specialists to name a quarter of the plants in this group. Without an orderly taxonomy it is impossible to use these plants for ecological, plant geographical, and other biological work. Verdoorn (1950) goes on to say, "whatever one may think about the relations between taxonomic and general biology, the taxonomists have to inventorize the resources of any region critically before other botanists will be able to work there well and successfully."

The following investigation is a taxonomic study of the mosses belonging to the family Pterobryaceae as they occur in the Southern United States, Mexico, Central America, and the West Indies. The 33rd parallel of latitude marks the northern limits of the group in the United States.

The family Pterobryaceae was first proposed by N. C. Kindberg in his *Studien über die Systematic der pleurokarpischen Laubmoose* in 1899, but references to mosses belonging to this group appeared in the literature long before that time. Hans Sloane (1707), and later Dillenius (1741) mentioned "the square branched *Hypnum* from Jamaica" (now *Orthostichopsis tetragona* (Hedw.) Broth.) as being not uncommon in Jamaica. Both of these authors illustrated the species. Olaf Swartz in his *Prodromus of the Mosses of the West Indies* (1788) referred to this moss as *Hypnum tetragonum* and collected it "on trunks of trees near summits of mountains in Jamaica." In the same volume, he briefly described *Fontinalis filicina* (now *Pireella filicina* (Hedw.) Card.) as also occurring in Jamaica. These references are the only pre-Hedwigian accounts of mosses which were later to become members of the Pterobryaceae.

Hedwig (1801) included a description and figures of *Hypnum tetragonum* Swartz, and also of *Fontinalis filicina* Swartz which he placed under the genus *Neckera*. Bridel (1822) likewise included these species under *Hypnum* and *Neckera*, which by this time had already become the chief "dumping grounds" for most pleurocarpous mosses. Christianus F.

Hornschuch, in Martius' *Flora Brasiliensis* (1840), established the genus *Pterobryon*[1] and made the combination, *Pterobryon densum* for Schwaegrichen's species, *Pterogonium densum* (1828). Hornschuch placed *Pterobryon* under the section *Hypnoidei.*

C. Müller (1851) in the *Synopsis Muscorum Frondosorum* devised a more elaborate and detailed classification, but kept most of the species in the tribe *Hypnoideae* as members of *Neckera,* and as a subsection of this genus established *Orthostichella* to include those plants with smooth cells and seriate leaves. Hornschuch's genus *Pterobryon* is maintained as a section under *Neckera* to comprise mosses with a frondose habit, creeping primary stems, pinnate branching, concave, plicate, or smooth leaves with one or two costae and immersed capsules. *Pterobryon hornschuchii* C. Müll. listed in this section is a synonym for *P. densum* (Schwaegr.) Hornsch., while *P. augustifolium* C. Müll. has been transferred to the genus *Pireella* as it is now understood. *Pterobryon densum* is placed by Müller in the section *Cyrtopus* proposed by Bridel (1822). At this point it becomes apparent that *Pterobryon* might be considered the type genus of the Pterobryaceae, and was for many years to be the reservoir where most pterobryaceous mosses were placed.

Mitten (1869) in his outstanding classic *Musci Austro-American* included *Pterobryon* in the tribe Neckereae, and divided the genus into four sections:

Section I. *Eupterobryum* Mitt. Dendroid species with immersed capsules. *Pterobryum densum* (Schwaegr.) Hornsch. *P. filicinum* (Sw.) Mitt. *P. trichomanoides* (Spruce) Mitt. *P. augustifolium* (C.M.) Mitt.

Section II. *Orthostichella* C.M. Mosses with seriate leaves and capsules exserted on a long seta. *P. pohlii* (Schwaegr.) Mitt. *P. cymbifolium* (Sull.) Mitt.

Section III. *Cyrtopus* Brid. Mosses with a dendroid habit, leaves radially arranged or equally disposed on the stem; capsules exserted. *P. brasiliense* (Hornsch.) Mitt.

Section IV. *Calyptothecium* Mitt. Stems elongated, distichous, and pinnate. *P. duplicatum* (Schwaegr.) Mitt.

This arrangement shows a considerable improvement over any of the earlier ones. At the present time the single species in the section *Cyrtopus* is included in the Pilotrichaceae and the genus *Calyptothecium* has been transferred to the Neckeraceae. In the *Adumbratio Florae Muscorum* by Jaeger & Sauerbeck (1877-78), *Pterobryon* was also included in the tribe !Neckeraceae, but the latter was put into the family Pilotrichellaceae, along with *Papillaria* C.M., and *Pilotrichella* C.M. The latter two genera of pendent mosses are now recognized as members of the Meteoriaceae, a family closely related to the Pterobryaceae. Unfortunately, Jaeger & Sauerbeck added no descriptions to their lists of generic and family names.

Between the years 1840 and 1898, several new genera were established, namely, *Jaegerina* C.M. 1876, *Renauldia* C.M. 1879, and *Orthostichidium* C.M. 1895, which were later to become part of the Pterobryaceae. During this interval these genera were jostled around from the Leucodontaceae, Neckeraceae, Pilotrichaceae, and Endotrichaceae, but most frequently were included as members of either the Hypnaceae or Neckeraceae. It was not until the publication of Kindberg's *Studien über die Systematik der pleurokarpischen Laubmoose* (1899) that the species of the Pterobryaceae were

1 *Pterobryon* has been consistently incorrectly spelled as *Pterobryum* by bryologists for 110 years. A detailed account of this matter is given following the generic description of *Pterobryon* (see pp. 20-21).

first critically studied, and sporophyte characters were used as diagnostic features. Kindberg's selection of the name Pterobryaceae for this family of plumose, fern-like, or dendroid mosses is a fortunate one, for it clearly describes the growth habit of the majority of species occurring in this group. Kindberg preceded his classification of the Pterobryaceae with the statement that he would risk an attempt to define the genera known to him, although he was not in the position to give a complete description. His outline of the family is translated below:

PTEROBRYACEAE

Leaves without marginal cells, the cells smooth; apical cells small and elongated. Peristome double or single; calyptra cap-shaped. Capsules generally immersed. Stems generally dendroid.

A. Peristome double. Capsule immersed or exserted (in *Orthostichella*).
  a. Costa single and long.
    Pterobryon Hornsch. Leaves narrow, often plicate; alar cells few or indistinct. Capsules immersed.
    Orthostichella C.M. Leaves short, broad, not plicate; alar cells numerous; capsules exserted.
  b. Costa short and double, or indistinct.
    aa. Leaves serrate; stems often flattened.
      Calyptothecium Mitt. Leaves two-ranked; alar cells few.
      Trachyloma Brid. Leaves almost two-ranked; alar cells numerous, transparent.
      Garovaglia Endl. Leaves radially arranged on the stems; alar cells few or indistinct.
    bb. Leaves entire; stems not flattened.
      Hildebrandtiella C.M. Alar cells numerous, enlarged, opaque.
B. Peristome single.
  a. Capsules immersed; leaves entire.
    Orthostichidium C.M. Basal leaf cells enlarged, yellow-brown. Alar cells indistinct. Operculum not attached to columella.
    Wardia Harv. Alar cells numerous, enlarged, transparent. Operculum attached to columella.
  b. Capsules exserted; leaves serrate.
    Oedicladium Mitt. Basal leaf cells yellow-brown; alar cells indistinct.

Since the appearance of this publication, several changes in the arrangement of the mosses in this family have been made. The genus *Wardia* Harv. has been transferred to the Fontinalaceae, *Orthostichella* C.M. has been divided into several genera, and both *Squamidium* (C.M.) Broth. and *Pilotrichella* (C.M.) Besch., are included in the Meteoriaceae. Mitten's genus *Oedicladium* is now *Myurium Schimp.* which along with *Piloecium* C.M. comprise the family Myuriaceae. *Calyptothecium* Mitt., as has been mentioned, is a genus in the Neckeraceae. The genera *Trachyloma* Brid., *Garovaglia* Endl., and *Hildebrandtiella* C.M. are retained in the Pterobryaceae but none of these is American: *Garovaglia* is eastern Malayan-Polynesian, *Trachyloma* occurs in Ceylon, New Guinea, Polynesia, and Australia, and *Hildebrandtiella* is distributed along the east coast of tropical Africa and Madagascar.

Six years after Kindberg's publication, Fleischer (1901) published a very thorough, expanded classification of the Pterobryaceae, which was for the most part repeated in his revolutionary and completely original work *Die Musci der Flora von Buitenzorg* (1900-1922). He rearranged Kindberg's earlier classification, and divided the family into four tribes to include all the genera known at that time. In the classification translated from the German, which follows, the genera occurring within the area considered in this study are indicated by an asterisk:

PTEROBRYACEAE (Kindberg) Fleischer

I. Tribe Oedicladieae Fleischer.—Plants caespitose, forming mats; leaves concave, smooth, ecostate. Seta elongated. Calyptra hood-shaped. Exostome with perforations.

Genus: *Myurium* Schimp. (*Oedicladium* Mitt.)

II. Tribe Pterobryeae Fleischer.—Plants growing in lax groups, seldom in dense tufts; capsules often immersed, seldom exserted; calyptra cap-shaped, rarely cucullate. Exostome smooth, often with thickenings, usually with transverse ridges; segments generally absent, seldom transversely striate.

Genera: **Jaegerina* C.M., *Spiridentopsis* Broth., **Pterobryopsis* Fleischer, *Symphysodon* Dz. & Molk., *Symphysodontella* Fleischer, **Renauldia* C.M., **Orthostichopsis* Broth., *Rabdodontium* Broth., **Pirea* Card., (now *Pireella* Card.), *Müllerobryum* Fleischer, **Pterobryum* Hornsch., *Pterobryella* C.M.

III. Tribe Garovaglieae Fleischer.—Plants growing in tufts; primary stems short, matted with rhizoids. Capsules immersed; calyptra usually cucullate; exostome usually papillose, segments generally absent.

Genera: *Garovaglia* Endl., *Endotrichella* C.M., *Euptychium* Schimp.

IV. Tribe Trachylomeae Fleischer.—Plants forming dense tufts; primary stems long, partly matted with rhizoids. Seta somewhat elongated. Calyptra cucullate, pilose. Exostome papillose, segments short.

Genus: *Trachyloma* Brid.

The tribe Oedicladieae Fleischer has since been discarded and the species have been placed in a new family, the Myuriaceae, with two genera *Myurium* Schimp. and *Piloecium* C.M. The Myuriaceae as thus composed is widely distributed in the Netherlands East Indies, Japan, Korea, Java, Borneo, and the Philippine Islands. The tribes Garovaglieae and Trachylomeae appear to comprise natural and distinct groups. The remaining tribe, the Pterobryeae is a very distinct and natural segregation in which the characteristic tendencies of the group are exhibited in varying degrees by the genera and species.

Brotherus (1906), did not feel that the family was worthy of separate recognition and returned to the older concept of classification wherein the members of the Pterobryaceae were treated as the sub-family Pterobryeae, in the Neckeraceae. Later, however, in the second edition of the Musci (1924-1925) in Engler & Prantl's *Die natürlichen Planzenfamilien*, he adopted the classification of Fleischer except for the tribe Oedicladieae. Although Brotherus made few major changes in Fleischer's classification, he did incorporate several minor ones. Fleischer's tribes were assigned to subfamilies, and the tribe Oedicladieae was removed to the Myuriaceae. The arrangement of Brotherus is translated below:

PTEROBRYACEAE

A. Main stem thread-like, sparsely beset with shizoids; leaves absent or distant. Secondary stems devoid of paraphyllia. Dorsal layer of peristome teeth thickened and smooth, inner peristome fugacious, united to exostome, segments usually absent ..... Pterobryoideae

B. Main stem rhizomatous, thickly beset with brown hairs or rhizoids. Dorsal layer of peristome normally developed, seldom smooth. Inner peristome free, basal membrane and segments present.

a. Secondary stems dendroid, provided with subulate paraphyllia; costa stout, percurrent or excurrent ..... Pterobryelloideae

b. Costa lacking or very short.

1. Secondary stems provided with a few appressed leaves below; branches flattened; paraphyllia present or absent ..... Trachylomoideae

2. Secondary stems usually simple, seldom distantly or irregularly branched, sometimes slightly flattened, leafy; paraphyllia absent ..... Garovaglioideae

The foregoing arrangement, which is primarily that of Fleischer, represents the most modern interpretation of the Pterobryaceae. However, a short time later, T. R. Sim (1926) in a study of the *Bryophyta of South Africa* adopted some of the older ideas and included the genera *Renauldia* C.M. and *Pterobryopsis* Fleischer, the only two considered in his work, in the subfamily Pterobryeae, family Neckeraceae.

At this point it becomes apparent that the members of the Pterobryaceae have had a long confused history in which the main groups and genera have been reshuffled and interpreted in a variety of ways. As far as the plants occurring in this area are concerned, the groupings of genera and species made by earlier workers were in many instances purely artificial assemblages based on characteristics so variable that one genus or species might be included in several sections.

The classification of the American members of the family as here revised, based particularly on a study of the species occurring in the southern United States, Mexico, Central America, and the West Indies, follows in a general way Fleischer's works (1900-1922; 1906) and includes the following sub-divisions:

PTEROBRYACEAE (Kindberg) Fleischer

I. Tribe Pterobryeae Fleischer.

Genera: *Jaegerina* C.M., *Pireella* Card., *Pterobryon* Hornschuch, *Pterobryopsis* Fleischer, *Orthostichidium* C.M., *Orthostichopsis* Broth., *Renauldia* C.M., *Spiridentopsis* Broth. (Brazil).

II. Tribe Garovaglieae Fleischer (no American representatives).

III. Tribe Trachylomeae Fleischer (no American representatives).

Illustrations and descriptions have been drawn from type material, except when it was in too poor condition or in too scanty amounts. The names and locations of the various herbaria from which specimens have been examined, and the standard symbols used in referring to them throughout this study follows: MICH—University of Michigan, Ann Arbor; NY—New York Botanical Gardens, New York; UC—University of California, Berkeley; MO—Missouri Botanical Gardens, St. Louis; Y—Yale University, New Haven, Connecticut; F—Chicago Natural History Museum, Chicago, Illinois; TENN—University of Tennessee, Knoxville; FH—Farlow Herbarium, Cambridge, Massachusetts; US—National Herbarium, Washington, D. C.; PC—Muséum National d'Histoire Naturelle, Paris, France; BART—E. B. Bartram, Bushkill, Pennsylvania; DUKE—Duke University, Durham, North Carolina.

ACKNOWLEDGEMENTS

The author is indebted to the following for use of herbarium specimens: Mme. V. Allorge and Mme. S. Jovet-Ast of the Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle, Paris, France; E. B. Bartram; The New York Botanical Garden; University of Michigan; University of Tennessee; University of California; Missouri Botanical Garden; Yale University; Harvard University; Chicago Natural History Museum, and the United States National Herbarium. Especial appreciation goes to Dr. R. J. Lowry, Dr. Margaret Fulford, Dr. A. H. Smith, Dr. F. K. Test, and Dr. E. U. Clover for valuable advice throughout, and to Dr. W. C. Steere, under whom this study was begun.

Grateful acknowledgment is made to the University of Michigan Biological Station for research facilities provided for over a period of three years, and to the Horace H. Rackham School of Graduate Studies of the University of Michigan for a University Fellowship during 1949-50, and University Scholarships in the summers of 1949 and 1950.

PTEROBRYACEAE (Kindberg) Fleischer

Plants medium size to robust, rarely small, often very showy, glossy or dull, dendroid or frondose, densely tufted forming thick mats on bark (rarely on rocks), frequently

pendent on twigs or branches; widespread in the tropical and sub-tropical parts of the world.

Primary stems filiform, short or long creeping, sparsely to thickly beset with reddish brown tomentum; leaves generally absent, when present, very small and scale-like; secondary stems solitary, irregularly pinnate or twice-pinnate, dendroid, often forming a woody stipe below, upright to pendent, never "rooting," usually without paraphyllia; stem in cross section round, central strand absent, composed mostly of thick-walled, pitted cells, outer row stereid; flagelliform branches often present.

Leaves one layer of cells thick, symmetrical, radially arranged on the stem, spreading, frequently spirally seriate, all alike or stem leaves larger than the branch leaves, oblong-angustate to ovate-lanceolate, short or long pointed, concave, smooth or plicate; costa slender, generally short, single, double or lacking, seldom percurrent or excurrent; leaf cells smooth or sometimes bearing a single, small papilla on each end wall, often porose and incrassate, rhomboidal to long linear in outline, the basal cells usually yellowish brown; alar cells usually differentiated.

Dioicous. Male and female plants the same size, the inflorescences similar in appearance, borne on usually erect, terminal or lateral, short, secondary branches; perichaetia large, the perichaetial leaves differentiated from ordinary leaves, often larger; perigonia small, the perigonial leaves usually smaller than the ordinary leaves; bud-shaped dwarf males sometimes produced on branch leaves (in the tribe Garovaglieae).

Capsules usually urn-shaped, smooth, erect, immersed, or exserted; stomata superficial, few, generally absent; annulus generally absent; operculum conic-rostrate; calyptra small, mitriform or cucullate, smooth or pilose; seta usually less than 10 mm. in length, smooth, rarely scabrous; peristome single or double, deeply inserted under the mouth, the 16 teeth of the exostome lanceolate, somewhat hygroscopic, sometimes perforated in the center, smooth, (rarely papillose), the ventral side sometimes with indistinct cross striae or dots; basal membrane absent; endostome generally rudimentary; segments slender, long or short, inarticulate, not keeled, often absent; a preperistome of a few segments sometimes present; spores polymorphous, smooth or papillose.

Gemmae frequent, septate, uniseriate, 3-16 cells long, borne in the axils of the leaves.

All species of the family in the area under consideration fall into the tribe Pterobryeae which is next described.

Tribe PTEROBRYEAE Fleischer

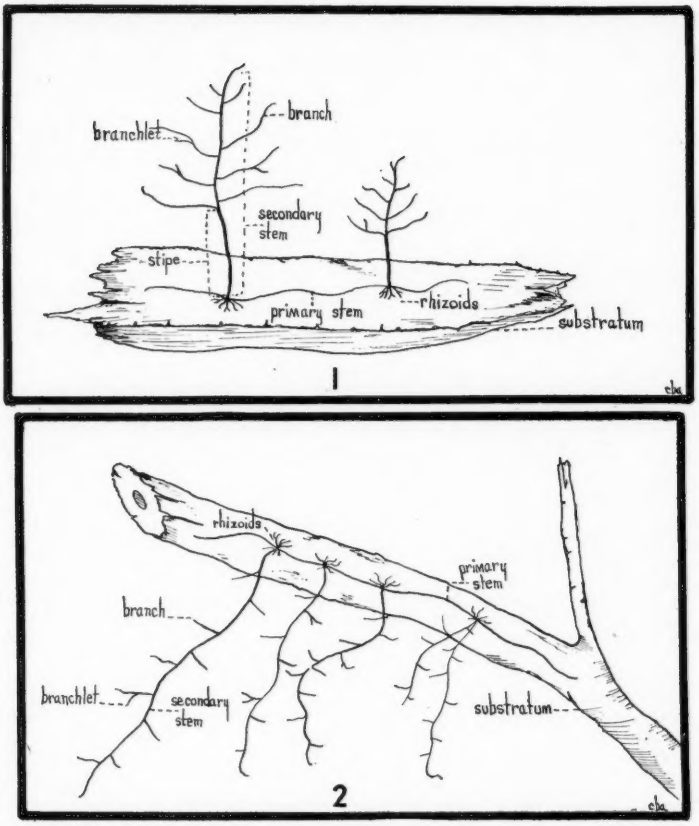
Plants slender to very robust, glossy, plumose, generally densely tufted, on bark, twigs or rocks, sometimes pendent; primary stems long, creeping, filiform, naked, or rarely bearing appressed scale-like leaves, reddish brown tomentum rarely present; secondary stems pinnate or twice-pinnate, pendent or dendroid, often stipitate below.

Leaves ovate-lanceolate to narrowly lanceolate, often cymbiform, concave, sometimes plicate; costa single, double, or lacking, rarely excurrent; leaf cells linear in outline, seldom short and incrassate, smooth or each end wall bearing a single, minute papilla, often porose; basal cells enlarged and colored; alar cells differentiated.

Capsules immersed or exserted, oval to oblong; seta short, seldom elongated; peristome usually double, rudimentary, a preperistome sometimes present, exostome smooth, often irregularly thickened; endostome fugacious, sometimes united to the exostome, segments generally absent; preperistome when present composed of a few short segments; calyptra mitriform or cucullate, naked or pilose.

Gemmae present or absent.

The terminology used in referring to the various parts of the plants in this family has been confusing, so that it has often been difficult to determine whether the reference to a main stem was actually to the primary stem or the secondary stem. In a like manner, whenever branches were mentioned, it was questionable whether they arose from the primary or the secondary stems. The primary stem is a long, creeping, filiform stem which is usually naked, or rarely bears minute scale-like leaves. It is seldom present on herbarium specimens, for it is usually left on the substratum



Figs. 1, 2.—1. Dendroid branching pattern in such genera as *Pireella* and *Pterobryon* illustrating the terms used in referring to various structures; 2. Pendent branching pattern in such genera as *Orthostichopsis* and *Orthostichidium*, illustrating terms used in referring to various structures.

when the plants are collected. Any innovation arising from the primary stem is a secondary stem; it may be dendroid as in *Pireella* and *Pterobryon* (see fig. 1), long pendent and branched as in *Orthostichopsis* and *Orthostichidium* (see fig. 2), or erect to tufted and unbranched as in *Jaegerina*. While *Renauldia* and *Pterobryopsis* commonly have pendent or tufted secondary stems, they may, on rare occasions, be dendroid. Branches are those innovations arising from the secondary stems, while branchlets develop from the branches. In typically dendroid or stipitate genera such as *Pireella* and *Pterobryon*, a stipe is that part of the secondary stem from the base up to the first set of branches (see figs. 1, 2).

KEY TO THE GENERA OF PTEROBRYACEAE OCCURRING IN THE SOUTHERN UNITED STATES, MEXICO, CENTRAL AMERICA, AND THE WEST INDIES

1. Costa short, double or lacking 2
1. Costa single 4
2. Branch leaves spirally seriate ..... 1. *Orthostichidium*
2. Branch leaves not in rows 3
3. Leaves squarrose, broadly ovate to lanceolate ..... 2. *Jaegerina*
3. Leaves loosely appressed to spreading, ovate ..... 3. *Renauldia*
4. Plants pendent, lax in appearance 5
4. Plants frondose or dendroid, rarely loosely tufted 6
5. Branch leaves strongly spirally seriate ..... 4. *Orthostichopsis*
5. Branch leaves weakly ranked; leaves concave, closely imbricated ..... 5. *Pterobryopsis*
6. Leaves plicate; capsules immersed ..... 6. *Pterobryon*
6. Leaves plane; capsules seldom immersed 7
7. Leaves erect spreading, spirally seriate ..... 7. *Pireella*
7. Leaves closely imbricated, rarely in distinct rows ..... 5. *Pterobryopsis*

DESCRIPTION OF GENERA AND SPECIES

1. ORTHOSTICHIDIUM C.M., in Dusén, K. Vetensk. Akad. Handl. 28(2):19. 1895.

Meteorium Sect. 1. *Eumeteorium* Mitt., Linn. Soc. London, Journ. Bot. 12:427. 1869. in part. *Pilotrichum* Hampe, Bot. Zeit. 27:449. 1869. in part. *Pilotrichella* Jaeg., in Jaeg. & Sauerb. Ber. St. Gall. Nat. Ges. 1874-75:161. 1876. in part. *Hildebrandtiella* Paris, Index Bryol. ed. 1. 1:948. 1896. in part.

Plants slender to robust, glossy, golden green or bright green, forming tangled, sometimes pendent masses or lax tufts on twigs and branches; secondary stems irregularly pinnate, elongated, generally numerous.

Leaves imbricated in spiral rows (seriate), concave, ecostate, smooth, ovate-lanceolate; apex acute to short acuminate, margins entire or serrulate; cells linear, the wall often sinuous, smooth, porose, yellowish brown, the alar cells differentiated, often numerous, small, subquadrate to rhomboidal, forming a well defined group (sometimes indistinct in our species).

Dioicous. Male and female inflorescences borne on short lateral branches; perichaetial leaves erect, costate or ecostate, usually larger than the ordinary leaves, gradually narrowed into a long slender point; the perigonial leaves smaller, generally ecostate; capsules immersed, oval to oblong-oval, light brown, the seta very short; peristome teeth narrowly-lanceolate, yellow, smooth, a preperistome present, endostome sometimes lacking; operculum conic-rostrate, short; spores from 38-40 μ, smooth; the calyptra mitriform, smooth.

Gemmae frequent, borne on the stems in the axils of leaves, cylindrical, hyaline, uniseriate, 2-12 cells long.

Type species.—*Hildebrandtiella perpinnata* Brotherus.

The genus *Orthostichidium* was established by Carl Müller (1895) to include two mosses from the west coast of Africa (*O. perpinnatum* (Broth.) Dus. and *O. cameruniae* C.M.) which he had formerly placed in his genus *Hildebrandtiella*. The latter genus bears a strong superficial resemblance to *Orthostichidium*, but is readily distinguished by fundamental differences in such sporophyte characters as the long beaked operculum, the relatively simple construction of the peristome, and the cucullate calyptra. In the gametophyte, it is distinguished by the large group of comparatively well defined alar cells.

Orthostichidium is clearly distinctive in having a laxly tufted to pendent growth habit, a glossy green color, and leaves which are ecostate, and arranged in five series on the secondary stems. *Orthostichopsis* Broth., is easily separated by its generally very lax appearance, narrower, long pendent secondary stems, and costate leaves ending in a short mucro or long filiform point. The leaves in the common *Orthostichopsis* species are plicate.

Orthostichidium is a tropical genus containing perhaps a dozen species. A thorough study of these is greatly needed before any general statements can be made in regard to the relationships of the species. In the area under consideration, the genus is represented by a single species.

ORTHOSTICHIDIUM PENTAGONUM (Hampe & Lor.) C.M.,
Bull. Herb. Boiss. 5:205. 1897

Pilotrichum pentagonum Hampe & Lor., Bot. Zeit. 27:449. 1869 [July]. *Meteorium excavatum* Mitt., Linn. Soc. London, Journ. Bot. 12:430. 1869 [Dec.]. *Pilotrichella pentagona* (Hampe & Lor.) Jaeg., in Jaeg. & Sauerb. Ber. St. Gall. Nat. Ges. 1875-76:161. 1876. *Hildebrandtiella pentagona* (Hampe & Lor.) Paris, Index Bryol. ed. 1. 1:948. 1896. *Orthostichidium subtetragonum* C.M., Bull. Herb. Boiss. 5:205. 1897. *Orthostichidium excavatum* (Mitt.) Broth., in E. & P. Nat. Pflanzenf. ed. 1. 1(3):795. 1906.

Plants growing in glossy, golden green, loose tufts, of pendent masses from branches and twigs of trees and shrubs; primary stems creeping, usually naked, leaves when present minute, 0.5 mm. long, lanceolate, closely appressed; secondary stems 4-8 cm. long, spreading and irregularly pinnate, the branches distant, 1.3 cm. long; leaves densely imbricated, disposed in five spiral rows, 1.5-3 mm. long, 0.8-1.5 mm. wide at the base, concave, ecostate, entire, or rarely serrulate above, plane, ovate-lanceolate, margins broadly involute above; apex acute to short acuminate; median cells 40-67 μ x 2-4μ, smooth, porose, the walls often sinuous; cells of the leaf apices shorter, rhomboidal, ranging from 4-18 μ x 4 μ; basal cells short and yellowish brown across the insertion, 11-22 μ x 4-6 μ; alar cells few, in a small often poorly defined group, irregularly rounded to quadrate, 11-22 μ in diameter.

Dioicous. Male and female inflorescences borne on short lateral branches, the perichaetial leaves usually larger than the ordinary leaves, reaching 3.5 mm. in length, ecostate, gradually tapering into a long slender point, areolation the same as in branch leaves; perigonial leaves smaller than ordinary leaves, ovate, acuminate, ecostate; capsules immersed, oval to oblong-oval, 2.5-3 mm. long, 1.5 mm. wide, light brown; seta very short, 0.8-1 mm. in length, smooth, peristome double; teeth of the exostome narrowly lanceolate, hyaline, smooth, cilia nodulose; endostome composed of a few short segments; operculum short, conic-rostrate; spores smooth, polymorphous, averaging 40 μ; the calyptra mitriform, smooth.

Type locality: Ecuador: Loja (no data) on trunks of trees in woods, Timbara, 3400 ft. alt., *H. Krause.*

General distribution: Mexico, Costa Rica, West Indies, and South America.

Illustrations: Bart., Mosses of Guatemala, *f.* 113, F-G; Plate 1.

Mexico. VERACRUZ, near Jalapa (on old trees in dense forests), 4000 ft. alt., July 7, 1908, *C. G. Pringle Plantae Mexicanae* 10829 (NY, Y, US, FH, MICH).

Central America. GUATEMALA: Escuintla, between Rio Jute and Rio Pantaléon, on road between Escuintla and Santa Lucía Cotz, 540-720 m. alt., Jan. 24, 1939, *P. C. Standley* 63393 (F, MICH, HART). EL SALVADOR: Summit of Cerro del Guayabal (as *O. excavatum*) 1440 m. alt., Jan. 3-4, 1924, *Calderon* 1996 (NY), 1996a (US). NICARAGUA: Managua, vicinity of Casa Colorada near El Crucero, summit of Sierra de Managua (on tree trunk), 800-900 m. alt., May 14-25, 1947, *P. C. Standley* 8152 (F, MICH, BART). COSTA RICA: Alajuela, Colinas San Pedro de San Ramon, Dec. 28, 1932, *A. M. Brenes* 16694 (NY, F).

West Indies. HAITI: Ravine N.W. of Marmelade, 2230 ft. alt., Aug. 3, 1905, *G. V. Nash & N. Taylor* 1354 (NY, F, US). CUBA: Serra del Jaquey (In saxis humidis in sylvis) 2000 ft. alt., 1889 (NY). PUERTO RICO: Road north of Mayaguez, Mar. 4-12, 1906, *E. G. Britton & Delia W. Marble* (NY), July 19, 1915, *Stevens* 1863 (NY). TRINIDAD: Mount Tocuche, Apr. 3-5, 1920, *E. G. Britton, Dorothy Coker, W. Rowland* 1439 (NY, US, FH).

ECUADOR: Ad truncos in sylv. Timbara, 3400 ft. alt., Prov. Loja collecti. "Musci frondosi a cl. H. Krause in Ecuador." *Type* (NY).

O. pentagonum is very constant in its distinguishing characters. The ecostate leaves, which are conspicuously five-seriate on the stems, and the

glossy green gametophytes render it easy for recognition. It is sometimes confused with *Orthostichopsis tetragona* (Hedw.) Broth. from which it is readily distinguished by its plane, ecostate leaves, and the much coarser texture of the plants when moist. The usually erect, laxly tufted habit also marks it as distinct from *Orthostichopsis*.

O. pentagonum seldom varies in habit. Occasional gametophytes may have leaves distant, rather than closely imbricated, and the secondary stems may be laxer and more elongated. On some the secondary stems may be shorter and more compactly tufted, with the leaves much more crowded. The latter condition appears to be the most frequent variation of the two. Flagelliform branches have not been observed.

A comparison of the type of *Meteorium excavatum* Mitt. with that of *O. pentagonum* (Hampe & Lor.) C.M. (*Pilotrichum pentagonum* Hampe & Lor.) showed that the two plants are identical. The differences pointed out by Müller between *O. pentagonum* and *O. subtetragonum* C.M. are not constant. In the original description of *O. subtetragonum*, he explains that "*Orthostichidio pentagono* (Hpe. at Lor.) ex Ecuador simillimum, sed haecce species differt jam statura longiore graciliore foliisque apice involutaceo recurvis." An examination of the original material of *O. subtetragonum* shows that intergradations from long loosely branched stems to compact branches may be found in the same tuft of plants. Since the species *O. subtetragonum* was originally erected on these characters it is reduced to synonym.

2. JAEGERINA C.M., Linnaea 40:274. 1876

Hypnum Brid., Musc. Recent. Suppl. 4:184. 1819. *Pilotrichum* Sect. IV. *Cyrtopus* C.M., Syn. 2:158. 1851. in part. *Pterobryum* Sect. III. *Cyrtopus* Mitt., Linn. Soc. London, Journ. Bot. 12:425. 1869. *Garovaglia* C.M., Hedwigia 34:128. 1895. in part. *Pterobryopsis* Fleischer, Hedwigia 45:60. 1906. *?Jaegerinopsis* Broth., in E. & P. Nat. Pflanzenf. ed. 2. 11:144. 1925.

Plants medium size to robust, 4-10 cm. long, dull to glossy, green or yellowish green, forming lax, spreading to erect tufts (rarely tangled masses) on twigs and branches; primary stems creeping, slender, usually naked, leaves when present minute, scale-like; secondary stems simple or very sparingly branched, sometimes upright; flagelliform branches often present; leaves squarrose to widely spreading, often plicate, concave, ovate to lanceolate from a cordate base, margins entire or serrulate above, costate or ecostate; apex acute to long acuminate; cells linear, smooth, the walls porose, the alar cells small, often poorly defined, yellowish brown.

Dioicous. Perichaetia borne on short lateral branches, the perichaetial leaves larger and narrower than the branch leaves, costate or ecostate, ending in a long acuminate point; capsules exserted, erect, oval to oblong-oval, light brown, the seta usually short, 5-8 mm. in length, reddish, smooth, annulus often large and adherent to the operculum; peristome single, the teeth often short, narrowly to broadly lanceolate, obtuse, smooth or sometimes finely papillose, hyaline; operculum long beaked; spores smooth; the calyptra smooth or somewhat pilose.

Gemmae frequent, borne on the stems in the axils of leaves, cylindrical, often curved, long, uniseriate, 2-12 cells long.

Type species.—Jaegerina stolonifera C.M.

Jaegerina C.M. is a natural and distinct genus. The tufted, rarely branching secondary stems with squarrose leaves, together with the frequent presence of flagelliform branches allow it to be easily recognized.

Jaegerinopsis, a genus of doubtful validity, has no representatives occurring in the area here under consideration. The two species, *Jaegerinopsis*

scariosa and *J. squarrosa* formerly included in this genus have been transferred to *Jaegerina*. It should be understood that this transfer is confined only to those species within the area covered by this work. Such a change seems necessary because the species of *Jaegerinopsis* were separated from *Jaegerina* only on the basis of their broadly ovate leaves, whereas the leaves of *Jaegerina* were described as lanceolate. These distinctions seem to be trivial from a generic standpoint, especially when a careful study of leaf shape is made in the species of both genera. It is seen that on a secondary stem of *Jaegerinopsis*, broadly ovate leaves may intergrade with lanceolate leaves, and on a stem of *Jaegerina*, there may also be some leaves which are ovate in outline with an acute apex. A wide variation in leaf shape occurs on a single stem in both genera. Steere (1947), in *A consideration of the concept of genus in Musci* makes these comments, "One single character, even though a reasonably conspicuous one, should not be used alone to set up a segregate genus, or to evaluate an established one. New genera should be established only if they show a minimum of two constant characters in common, and preferably three."

KEY TO THE SPECIES OF JAEGERINA

1. Leaves narrow, tapering to a long slender point; cells porose in lower third of the leaf ..... *J. guatemalensis*
1. Leaves broad, ovate; apex acute to acuminate; cells porose throughout ..... *J. scariosa*

JAEGERINA GUATEMALENSIS Bart., Bryol. 49:116. 1946

Jaegerina jamaicensis E. G. Britton in Hb.

Plants robust, glossy, golden green, growing in loose mats; primary stems creeping, stoloniferous, slender, naked; secondary stems erect, 3-6 cm. long, simple or rarely with a few short branches up to 1 cm. in length, densely foliate; flagelliform branches bearing minute scale-like leaves 0.5 mm. long, frequent; leaves crowded, squarrose-spreading, 5.5 mm. long, 2 mm. wide at the base, concave, ovate-acuminate, apex tapering to a long slender point, cordate at the base, keeled above; margins often serrulate to near the base, sometimes entire; costa single, slender ending in the apex; cells long linear, more or less thick-walled, porose in the lower third of leaf, smooth or finely papillose at the apical angles, median cells linear, 40-70 μ x 5-8 μ, the cells of the leaf apices shorter, 28-38 μ x 5-15 μ, smooth, yellowish, the alar group poorly defined, composed of a few small, irregular to subquadrate cells 6-16 μ x 4-8 μ.

Dioicous. Capsules exserted, oblong-oval, 2 mm. long; seta erect, smooth, 8 mm. long; operculum long beaked, 1.5 mm. long; peristome single, the teeth narrowly lanceolate, hyaline, minutely papillose; spores brown, variously shaped, rounded to angular, 48-50 μ in diameter; the calyptra not seen.

Gemmae small, uniseriate, in axillary clusters, 3-15 cells long.

Type locality: Guatemala: Izabal, between Bananera and "La Prensa" in Montaña del Mico, 50-100 m. alt., *Steyermark* 39202.

General distribution: Guatemala and Jamaica.

Illustrations: Bart., Mosses of Guatemala, *f.* 112, *D-F;* Plate 2, fig. 1; Plate 3.

Central America. GUATEMALA: Izabal, between Bananera and "La Prensa" in Montaña del Mico, 50-150 m. alt. (epiphyte on tree bark, along upper part of slopes), Apr. 9, 1940, *J. A. Steyermark* 39202, *Type* (F, BART), 40-300 m. alt., Mar. 28, 1940, *J. A. Steyermark* 38205 (F, MICH).

West Indies. JAMAICA: Limestone Hills near Troy, Jan. 1910, *J. King Brown* 11173, 11183 (the type of *J. jamaicaicensis* E. G. Britton), (NY, BART).

As a rule it is fairly easy to distinguish this species from any other pterobryaceous plant, especially because of the general robust habit of the tufts, and the squarrose, long, gradually tapering leaves with a single costa ending in the long subulate apex. The cells are porose only in the lower

third of the leaf. The preceding characteristics will clearly separate it from *J. scariosa*, which is smaller in statute, with a shorter, ovate leaf terminating in an acute apex. *J. guatemalensis* exhibits only a small degree of variability in the length of the acumen and in leaf shape. The leaves may vary from long and narrowly lanceolate to ovate-acuminate in outline.

Jaegerina scariosa (Lor.) comb. nov.

Meteorium scariosum Lor., Moostudien, 151. 1864. *Pilotrichum scariosum* Lor. in Hb. *Garovaglia scariosa* (Lor.) C.M., Hedwigia 34:128. 1895. *nomen nudum*. *Pterobryopsis scariosa* (Lor.) Fleischer, Hedwigia 45:60. 1906. *Jaegerinopsis scariosa* (Lor.) Broth., in E. & P. Nat. Pflanzenf. ed. 1. 1(3):791. 1906. *Jagerinopsis squarrosa* E. G. Britton, Bryol. 21:48. 1918. *Jaegerinopsis cubensis* E. G. Britton in Hb.

Lorentz's original material was not available for study. His original description (1864) follows:

"Caulis primarius nudus (in lichene quodam) prorepens, ramos simplices, turgidos, aequales parvos emitens, qui, ubi substratum attingunt seu ex latere radices agunt et denuo caules prorepentes, nudos progignunt. Folia patentia, e basi angusta orbiculari-acuminata, concava, acumine brevi; nervi bini, brevissimi, margo planus, infra integerrimus, apice obsolete dentatus; rete breviter linerare, cellulae incrassatae, poris inter se conjunctae, basi tantum latiores et breviorea. Flores et fructus ignoti."

The following description was drawn from material collected since that time:

Plants slender, erect, bright green, glossy, forming lax tufts or masses on trees; primary stems creeping, slender, usually naked, leaves when present scale-like, 0.5 mm. long, lanceolate; secondary stems stout, erect, red, simple and unbranched, 1-2 cm. long, seldom to 4-5 cm., sometimes becoming flagelliform; leaves crowded, squarrose-spreading, broadly ovate to ovate-lanceolate from a cordate base, 1-3 mm. long, 1-1.5 mm. wide, rarely broader than long, concave, the apex variable, acute to acuminate, often recurved; leaf margins minutely and obscurely serrulate to base; costa variable, broadest at the base, single, or forking at apex, or sometimes short and double, usually ending below mid-leaf, rarely reaching the apex; cells narrowly long rectangular, smooth, thin walled, porose throughout, median cells 38-55 μ x 4-6 μ, those of the apical region usually shorter, 33-45 μ x 4-6 μ, the basal cells somewhat shorter, irregular in outline; alar group poorly defined, yellow, composed of a few subquadrate to irregular, incrassate cells 12-28 μ x 14 μ.

Dioicous. Perichaetia small, about 1 mm. long, forming small lateral buds on the branches, perichaetial leaves usually longer than ordinary leaves, acuminate, serrulate, ecostate; capsules exserted, oblong-oval, 2 mm. long, 0.6-0.8 mm. wide, light brown; seta short, 4 mm. long, smooth, reddish; operculum long beaked. Peristome, calyptra, and spores not seen.

Type locality: Panama, Chiriquí, April 1858, *M. Wagner*.

General distribution: Florida, Panama, Guatemala, Cuba, and the Dominican Republic.

Illustrations: Bart., Mosses of Guatemala, *f.* 112, *G-I;* Plates 2, 4.

United States. FLORIDA: Gadsden County (as *Jaegerinopsis squarrosa* E. G. Britton), Glen St. Julia (on *Magnolia virginiana*) Apr. 6, 1940, *Ruth O. Schornherst* 1576 (NY).

Central America.—GUATEMALA: Petén (as *Pterobryopsis scariosa* (Lor.) Fleischer), Fallabón-Yaxha Road, Mar. 22, 1933, *C. L. Lundell* 2044 (FH, MICH). COSTA RICA: Alajuéla (as *Jaegerinopsis scariosa* (Lor.) Broth.), 900 m. alt., Jan. 1910, *Oton Jiménez* 8 (NY). PANAMA: Chiriquí, *Type*, not seen.

WEST INDIES. CUBA: Pinar del Rio, S. Diego de las Banos, Sierra de las Yeguas (*Jaegerinopsis cubensis* E. G. Britton in Hb., and the type of *J. squarrosa* E. G. Britton), June 28, 1915, *Bro. Leon* 5239 (NY). DOMINICAN REPUBLIC: Vicinity of Piedra Blanca, La Vega, 200-500 m. alt., Dec. 17, 1947, *H. A. Allard* 179922 (MICH).

Although *Jaegerina scariosa* is allied to *J. guatemalensis*, the former appears to be quite distinctive as already pointed out in the specific discussion of *J. scariosa*.

There has been much confusion and diversity of opinion regarding the distinguishing characteristics of *Jaegerinopsis scariosa* and *J. squarrosa*,

which is no doubt to be explained because of the great variability in the number and length of the costae in both. Elizabeth Britton (1918), who first described *J. squarrosa* from Cuba, was of the opinion that *J. squarrosa* was distinct from *J. scariosa* and separated her species chiefly on the basis of the unicostate leaves, while *J. scariosa* from Panama had shortly bicostate leaves. Mrs. Britton explained that the leaves of *J. scariosa* were much more glossy and the cells of the apex of the leaves longer and narrower and much less porose, but that the alar cells of both species were very similar. The leaves of the latter species were also reported as being less spreading, and the costae more often double, though occasionally there was a leaf with a simple vein. In the specimens which I have examined of both species the costa exhibits a wide degree of variability. The leaves of the plants from Cuba and Florida are sometimes shortly bicostate or have the vein forking either at the base or the apex. Usually the costa is single and ends about the middle of the leaf. The costa sometimes shows this range of variation in leaves taken from a single stem. The degree of porosity of the cells and size of cells of the apex varies to a small degree from leaf to leaf, and in a like manner the glossiness of the plants may vary. The leaves are all squarrose to approximately the same degree, allowing, of course, for some slight individual variation in each leaf. It appears, therefore, that *J. squarrosa* should be included as a synonym of *J. scariosa* which antedates it by fifty-four years. Only one sporophyte of this plant has been seen, and that was in very poor condition with peristome and calyptra missing.

3. RENAULDIA C.M., *in* Renauld, Prodr. Fl. Bryol. Madag. 18:9. 1897

Cryptotheca Hornschuch, in Depp. et Schied. Musc. Mexic. [Exsiccati]. *Pilotrichum* C.M., Syn. 2:182. 1851. in part. *Meteorium* Mitt., Linn. Soc. London, Journ. Bot. 12:433. 1869. in part. *Calyptothecium* Mitt., *op. cit.* p. 312. in part. *Pterobryum* Jaeg., in Jaeg. & Sauerb. Ber. St. Gall. Nat. Ges. 1877-78:464. 1879. in part. *Pterobryopsis* Sect. I. *Pseudo-Calyptothecium* Broth., in E. & P. Nat. Pflanzenf. ed. 1. 1(3):802. 1906. in part.

Plants medium size to robust, 4-12 cm. long, bright green, seldom yellow-green, forming lax, scraggly mats or tangled masses on trees and branches; primary stems creeping, very slender, usually naked, the leaves when present scale-like; secondary stems irregularly pinnate, sparingly branched, pendent to dendroid, seldom erect; leaves crowded, spreading, weakly ranked, plicate, deeply concave, ovate-acuminate from a cordate base, apex acute to acuminate, margins entire or serrulate above, ecostate, or rarely bicostate to mid-leaf or below; cells long rectangular, smooth, porose, the cell walls more or less thickened, those of the alar region subquadrate to irregular in outline, yellow, forming an inconspicuous group.

Dioicous. Perichaetia large, borne on short lateral branches, the leaves larger than the ordinary leaves, lanceolate, ecostate; perigonial leaves smaller than branch leaves, ovate-lanceolate, ecostate; capsule immersed, broadly oval, light brown; peristome double, the teeth of the exostome narrowly lanceolate, hyaline, yellow, smooth or finely papillose, the endostome rudimentary; operculum conic-rostrate; spores polymorphous, often angular, smooth or finely papillose, 64 μ long, 32 μ wide; calyptra mitriform, smooth.

Type species.—Hildebrandtiella hildebrandtielloides Ren. & Card.

Müller (1897), in his study of the bryophyte flora of Madagascar, erected *Renauldia* to include those mosses differing from the genus *Hildebrandtiella* in having a mitriform calyptra, a double peristome, finely papillose teeth, and usually immersed capsules. The major distinctions between the two genera, therefore, are found in sporophyte characters, although the

comparatively well defined group of alar cells in *Hildebrandtiella* is a gametophytic feature which serves to separate that group from *Renauldia*.

Plants of *Renauldia* resemble those of *Pterobryopsis* and *Orthostichidium* in the similarity of the branching pattern. *Orthostichidium* is distinct in having conspicuously five-seriate leaves, while *Renauldia* and *Pterobryopsis* have leaves at best weakly ranked. Further, the ecostate leaves (sometimes faintly two nerved) and the poorly defined alar region in *Renauldia* contrast with the single, short, but well developed costa and large group of quadrate alar cells in *Pterobryopsis*. The general aspect of the two genera is fundamentally alike; although *Pterobryopsis* often takes on an erect, frondose habit, whereas *Renauldia* is more frequently matted or tufted.

RENAULDIA MEXICANA (Mitt.) Crum*

Cryptotheca cochlearifolia Hornschuch, ex Schlechtendal & Chamisso, Linnaea 6:62. 1831. *nomen nudum. Pilotrichum cochlearifolium* C.M., Syn. 2:182. 1851, non *P. cochlearifolium* C.M., Linnaea 17:599. 1843. *Meteorium mexicanum* Mitt., Linn. Soc. London, Journ. Bot. 12:433. 1869. *Pterobryum cochlearifolium* (C.M.) Jaeg., in Jaeg. & Sauerb. Ber. St. Gall. Nat. Ges. 1875-76:238. 1877. *Renauldia cochlearifolia* (C.M.) Broth., in E. & P., Nat. Pflanzenf. ed. 2. 11:146. 1925.

Plants robust, glossy, green to yellowish green, in lax tufts or mats on tree trunks; primary stems creeping, filiform, usually naked, leaves when present scale-like, lanceolate, ecostate, 0.5 mm. long; secondary stems suberect to pendent, sometimes dendroid, tumid, 4-8 cm. long, pinnately branched, the branches widely spreading to 1.5 cm. long; leaves crowded, closely imbricated, deeply concave, sometimes faintly plicate, those of the secondary stem reaching 5 mm. in length to 2 mm. in width; branch leaves generally smaller, 2-3.5 mm. long, 1-1.5 mm. wide at the base, secondary stem and branch leaves oblong-ovate from a subcordate base, abruptly short pointed, entire or serrulate above; costa double, very short, .23 mm. long, often absent; cells narrowly long rectangular, the walls more or less thickened, smooth, porose except in the alar cells, median cells 55-105 μ x 4-6 μ, those of the apical region of the same size or shorter, 40-98 μ x 4-10 μ, basal cells shorter and broader, 40 x 11 μ, lax, yellow at the insertion; alar cells few and inconspicuous, subquadrate to irregular in outline, 10-35 μ x 6-10 μ, thin-walled, porose, yellow.

Dioicous. Perichaetia borne on short lateral branches, large, to 4.2 mm. long, the perichaetial leaves 3.5-4 mm. long, ecostate, ovate-lanceolate, narrowed into a linear, acuminate, denticulate point; perigonial leaves smaller than the ordinary leaves, ecostate, lanceolate, apex acute; capsules immersed, broadly oval to oblong, 1.5 mm. long, 1-1.2 mm. wide, light brown; seta very short, 0.5 mm. long; operculum conic-rostrate, 0.4 mm. long; peristome double, the exostome teeth linear-lanceolate, hyaline, smooth or finely papillose, the endostome rudimentary, fugacious; spores polymorphous, oval to oblong, sometimes angular, smooth or minutely papillose, yellow, averaging 64 μ in length, and 32 μ in width; calyptra mitriform, smooth.

Type locality: Mexico: Deppe et Schiede, Musc. Mexic. [Exsiccatae].

General distribution: Mexico, Guatemala, Panama.

Illustrations: Bart. Mosses of Guatemala, *f.* 113, D-E; Plates 5, 6.

Mexico. MORELOS, on trees (as *Pterobryopsis cochlearifolia*), mountain canyon above Cuernavaca, 6500 ft. alt., Oct. 18, 1908, *C. G. Pringle Plantae Mexicanae* 10658 (NY, F, Y, MO, US, UC).

Central America. GUATEMALA: *Chimaltenango;* region of Los Positos, above Las Calderas (On tree trunk) 2250-2400 m. alt., Dec. 18, 1940, *P. C. Standley* 80167a (F, BART, MICH). PANAMA: Chiriquí, Boquete, on trail, Aug. 4-10, 1950, *A. M. Chickering* (MICH).

This infrequently collected plant is not easily confused with any other.

---

* The Appalachian-Ozarkian elements in the Mexican moss flora, with a check-list of all known Mexican mosses. Unpublished Ph.D. dissertation, 1951; microfilmed in the University of Michigan Library.

In habitat, *R. mexicana* resembles *Pterobryopsis mexicana* (Schimp.) Fleischer, in that both have weakly ranked tumid leaves, and a loose scraggly overall appearance. However, the similarity stops at this point. The single short costa and conspicuous alar region composed of many rows of quadrate cells in *P. mexicana* readily separates this species from *R. mexicana* which has leaves ecostate or shortly two nerved and poorly defined alar cells. The leaves and stems give the impression of being more tumid in *Renauldia,* a condition better observed than described. This is especially noticeable in freshly collected plants, particularly in the more erect growing forms, and in specimens which have not been pressed. In herbarium specimens which have been pressed flat this character is of course not noticeable, but it may be recovered more or less by immersion of the stems in water.

There has been considerable confusion in the nomenclature of this species partly because Müller used *Pilotrichum cochlearifolium* for two distinct plants; first in 1843 (Linnaea 17:599), to describe what is now recognized as *Pilotrichella flexilis* (Hedw.) Jaeg. in the Meteoriaceae, and again in 1851 (Syn. 2:182), for the species in question. The latter is considered to be the original description of *Renauldia mexicana* (Mitt.) Crum. Hornschuch never published the herbarium name *Cryptotheca cochlearifolia;* however, Schlechtendal & Chamisso (1831), listed the name but without a description. *Reuauldia cochlearifolia* (C.M.) Broth. is based on *Pilotrichum cochlearifolium* C.M. 1851, but the latter name was invalidated by an earlier homonym; *P. cochlearifolium* C.M. 1843, is a synonym of *Pilotrichella flexilis* (Hedw.) Jaeg. in the Meteoriaceae. Therefore, as Crum (1951) has pointed out, there is no alternative to taking up the next validly published specific epithet *Meteorium mexicanum* Mitt.

4. ORTHOSTICHOPSIS Broth., in E. & P., Nat. Pflanzenf. ed. 1. 1(3):804. 1906

Hypnum Hedw., Sp. Musc. Frond. 246. 1801. in part. *Isothecium* Brid., Bryol. Univ. II:377. 1827. *Pterigynandrum* Brid., Bryol. Univ. II:194. 1827. in part. *Neckera* Sec. VIII. *Pseudopilotrichum* Subsect. I. *Orthostichella* C.M., Syn. 2:123. 1851. in part. *Neckera* Sect. *Pilotrichella* a. *Orthostichella* Hampe, Vidensk. Meddel. naturhist. Foren. København. 141. 1879. *Meteorium* Sect. *Eumetorium* Mitt., Linn. Soc. London, Journ. Bot. 12:427. 1869. in part. *Pilotrichella* Jaeg., in Jaeg. & Sauerb. Ber. St. Gall. Nat. Ges. 1876-77:463. 1878. in part. *Hildebrandtiella* Paris, Index Bryol. ed. 2. 1:313. 1904. in part.

Plants slender to robust, glossy, golden green or bright green, usually forming long tangled masses on twigs and branches; primary stems creeping, filiform, usually naked, leaves when present 0.5 mm. long, scale-like, lanceolate, ecostate; secondary stems pendulous, numerous, elongated, pinnately branched, the branches short and generally narrowed to the apex, 1.4 cm. long; leaves concave, costate, erect or imbricated in spiral rows, plane or plicate, ovate-lanceolate to lanceolate, apex acute, mucronate, or ending in a long filiform point, the margins entire or serrulate; the costa single, slender, sometimes very faint, ending about mid-leaf, rarely percurrent; cells narrowly linear in outline, sometimes sinuous, smooth, porose, yellow and shorter at the insertion, the alar cells differentiated, small, subquadrate, often numerous, forming a well defined group.

Dioicous. Male and female inflorescences borne on short lateral branches; perichaetial leaves erect, costate, variable in size, usually larger than the ordinary leaves, gradually narrowed to a long slender point; the perigonial leaves smaller, usually ecostate, antheridia few; capsules immersed in our species, oval to oblong-oval, light brown, the seta very short; peristome double, the teeth narrow, lanceolate, smooth, a preperistome present, the

endostome rudimentary, united to theexostome, segments absent; operculum conic-rostrate; spores polymorphous, smooth, averaging 42 μ; the calyptra cucullate, pilose.

Type species.—*Hypnum tetragonum* Hedwig. (*Hypnum tetragonum* Swartz, pre-Hedwigian).

The genus *Orthostichopsis* is a very natural and distinct group of large, glossy, long-pendulous or laxly tufted mosses growing on trees or hanging from twigs and branches. Our species are readily identified by their immersed capsules, generally plicate and lanceolate leaves with a single, short, well defined costa ending at mid-leaf or little beyond. The cells are linear and porose; those of the alar region form a very conspicuous differentiated group. The genus resembles *Orthostichidium* in aspect, but the single species of the latter which occurs in Mexico, Central America, and the West Indies, has ecostate, plane, leaves and an inconspicuous group of alar cells. *Orthostichopsis* is badly in need of revision; approximately twenty one species are reported for South America and the old world tropics.

KEY TO THE SPECIES OF ORTHOSTICHOPSIS

1. Branch leaves abruptly short mucronate, plicate ..... *O. tetragona*
1. Branch leaves gradually narrowed into a long, filiform point, plane ..... *O. crinita*

ORTHOSTICHOPSIS TETRAGONA (Hedw.) Broth., *in* E. & P., Nat. Pflanzenf. ed. 1. 1(3):805. 1906

Hypnum tetragonum Hedw., Sp. Musc. 246. 1801. *Pterigynandrum quadrifarium* Brid., Bryol. Univ. II:194. 1827. *Isothecium tetragonum* Brid., Bryol., *op. cit.* 377. *Neckera quinquefaria* C.M., Syn. 2:124. 1850. *Neckera tetragona* C.M., *op. cit.* 125. *Meteorium tetragonum* Mitt., Linn. Soc. London, Journ. Bot. 12:431. 1869. *Pilotrichella tetragona* Besch., Mem. Soc. Sci. Nat. d. Cherbourg 16:223. 1872. *Hildebrandtiella tetragona* (Hedw.) Paris, Index Bryol. ed. 2:313. 1904.

Plants robust, glossy, golden green or brownish, pendulous, forming tangled masses on twigs and branches, rarely on soil; primary stems creeping, naked, leaves when present minute, about 0.5 mm. long, lanceolate, ecostate, closely appressed; secondary stems pendent, numerous, 5-10 cm. long, distantly pinnate; branches divergent, 1.5 cm. long; leaves of secondary stems densely imbricated, 2-3.5 mm. long, 1.5 mm. wide at base, not seriate, erect, ovate-lanceolate, plane, concave, abruptly contracted into a long, linear-subulate, flexuose point; margins denticulate to the base or at least above; costa single, slender, ending a short distance above mid-leaf; branch leaves distinctly imbricated in five spiral rows, to 2 mm. long, oblong-ovate, plicate, concave, abruptly short mucronate; margins entire or denticulate above; costa single, slender, ending well above mid-leaf; cells of the branch and secondary stem leaves long linear in outline, the walls more or less thickened, smooth, porose throughout; median cells 35-64 μ x 3-6 μ, often sinuous, the cells of the apical region shorter, 12-40 μ x 4-10 μ, the basal cells short, 20-40 μ x 8-18 μ, yellow, the alar group well defined, the cells numerous, incrassate, small, 9-23 μ x 8-20 μ, quadrate to transversely elongated, forming 8-12 conspicuous rows.

Dioicous. Male and female inflorescences borne on very short lateral branches; perichaetia large, 4 mm. long, the leaves 2.5-4 mm. long, narrowly lanceolate, plane, the margins denticulate or entire; costa ending a short distance beyond mid-leaf; areolation as in the ordinary leaves; perigonial leaves very small, to 0.5 mm. long, entire, ovate, acuminate, ecostate or the costa faint, vanishing in the apex; areolation the same as in ordinary leaves; capsules immersed, oval to oblong-oval, 2.5-3 mm. long, 1.5 mm. wide, light brown; the seta very short, 0.5-0.8 mm. in length, smooth; peristome double, the teeth of the exostome narrowly lanceolate, hyaline, smooth, the endostome rudimentary, fugacious; operculum conic-rostrate, the beak short, curved, sharp pointed; spores large, 64-77 μ, polymorphous, rounded to angular, yellowish, smooth or finely and irregularly reticulated; the calyptra cucullate, pilose.

Type locality: Jamaica: on trunks of trees, near summits of mountains. *Swartz.*

General distribution: Mexico, Central America, West Indies, and South America.

Illustrations: Hedwig. Sp. Musc. *pl.* 63. Bart., Mosses of Guatemala, *f.* 114, *A-C;* Plate 7.

Mexico. VERACRUZ, Jalapa (in moist virgin forest) 5000 ft. alt., Dec. 12-20, 1909, *W. A. Murrill & Edna L. Murrill* 12 (F).

Central America. GUATEMALA: Izabal, Cerro San Gil, along Rio Bonita (on branch), 30-150 m. alt., Dec. 21, 1941, *J. A. Steyermark,* 41745 (NY, F, US, FH, MICH, UC, BART). BRITISH HONDURAS: Toledo district, Monkey River, Dec. 7, 1941, *P. H. Gentle* 3848 (F, FH, MICH). HONDURAS: Bonaca Island, *G. F. Gaumer,* no data, 167 (F, NY). NICARAGUA: Cabo Gracias a'Dios, May 1, 1923, *Rev. F. E. Schram* (NY). COSTA RICA: Alajuela, San Pedro de San Ramon (on twigs), Jan. 3, 1933, *Alberto M. Brenes* 16890 (NY, F), Jan. 30, 1935, *Alberto M. Brenes* 20366 (NY, F). PANAMA: Cana (on tree trunk), June 7, 1908, *R. S. Williams* 1069 (NY, F).

West Indies. JAMAICA: On trunks of trees, near summits of mountains in Jamaica (*Hypnum tetragonum* and *Hypnum 4-gonum poteris 5-gonum*), *Swartz, Type* (NY). CUBA: Santa Clara, south of Sancti Spiritus, winter 1920-21, *Ant. Luna* 1000 (NY). DOMINICAN REPUBLIC: Santo Domingo, Llano Costero, El Nanielito (on the ground in shaded places), Jan. 22, 1929, *E. L. Ekman* 11294 (NY, F, US). MARTINIQUE: Source Sulfurense des Deux-Choux, 680 m. alt., Jan. 5, 1941, *M. & H. Stehle* 3872 (NY). GRENADA: Mirabeau, St. Davids (On mountain trees in woods), Dec. 23, 1905, *W. E. Broadway* (NY). TRINIDAD: Aripo Road *via* Aunia, Nov. 21, 1924, *W. E. Broadway* 7853 (NY).

The distinguishing characteristics of the species are the robust, glossy green, pendent gametophytes, the seriate, short pointed, plicate branch leaves, and the very conspicuous alar region of from 6-12 rows of incrassate, quadrate cells in secondary stem and branch leaves.

This is an exceedingly protean species, and the characters given in the specific description, especially as regards the size of the plants, modes of branching, and nature of leaf apices, must be looked upon as approximations only. One of the conspicuous trends of variation observed in material from the West Indies, but not entirely confined to this area, is that in which the apices of the leaves at the tips of the branches tend to become long-subulate so that they are similar to the secondary stem leaves. The leaves of this variant, however, remain stable in all other characteristics, except that they may be less plicate. Most of the other plants of the species fall within a less distinct, variable group. The stems may be short and tufted, less than five cm. long, with many thick branches, or they may be as much as ten cm. in length and distantly branched and pendulous. The original material of *O. tetragona* collected by Schwartz in Jamaica consists of no more than a fragment of a stem scarcely more than one cm. in length.

In spite of its variability, *O. tetragona* is rarely difficult to distinguish from the closely related species *O. crinita* (Sull.) Broth., which may always be recognized by its plane branch leaves ending in long hair-like points nearly as long as the rest of the leaf. The comparatively inconspicuous alar regions of these leaves, composed of only four to eight rows of transversely elongated or quadrate cells will further aid in separating the two species.

ORTHOSTICHOPSIS CRINITA (Sull.) Broth., in E. & P., Nat. Pflanzenf. ed. 1. 1(3):805. 1906

Neckera crinita Sull., U. S. Expl. Exped. 5. 1859. *Meteorium crinitum* Sull., U. S. Expl. Exped. 22. 1859. *Meteorium erinaceum* Spruce, Cat. Musc. n. 18. 1867. *Orthostichella crinita* Hampe, Vidensk. Meddel. Naturhist. Foren. København. 25:277. 1870.

Plants robust, pale green to golden green, glossy, growing in large, pendent or loosely entangled masses on twigs and branches; primary stems creeping, usually naked, leaves

when present minute, about 0.5 mm. long, lanceolate, ecostate, closely appressed; secondary stem pendent, numerous, 8-15 cm. long, distantly pinnate, the branches short, 1.3 cm. long, straight, cuspidate, often in fascicles of twos and threes, thicker than the secondary stem; secondary stem leaves densely imbricated, 1.8-4 mm. long, 0.8-1.5 mm. wide below, not seriate, erect, narrowly lanceolate, plane, concave, abruptly narrowed into a long linear, serrulate point nearly as long as the rest of the leaf; costa single, slender, ending abruptly at mid-leaf, or a little beyond; branch leaves erect, imbricated, weakly ranked to five-seriate, 2-4 mm. long, 1.5 mm. wide below, narrowly lanceolate, plane to weakly plicate, concave, abruptly narrowed into a long, flexuous, serrulate or entire, filiform point, 1-2 mm. long; costa single, narrow, ending at mid-leaf; cells of the branch and secondary stem leaves similar, long linear, the walls more or less thickened, smooth, porose throughout; median cells 35-65 μ x 3-6 μ, sometimes sinuous, the cells of the apical region shorter, 30-40 x 2-4 μ, the basal cells 20-34 x 6-10 μ, yellow, the alar group small, distinct, the cells small, subquadrate to irregular in outline, incrassate, 4-35 μ x 6-16 μ, forming 4-8 rows.

Dioicous. Male and female inflorescences borne on very short lateral branches; the perichaetia small, 1.5-2 mm. long, the leaves 1-2 mm. long, lanceolate, ending in a subulate point, plane, margins entire or denticulate above; costa faint, ending a little above mid-leaf; areolation as in the ordinary leaves; perigonial leaves very small, 0.5-0.8 mm. long, ovate, acuminate, entire, ecostate or the costa single, very faint, ending at mid-leaf; areolation as in the ordinary leaves; capsules immersed, oval to oblong-oval, 2.5-3 mm. long, 1.5 mm. wide, brownish; the seta very short, 0.5-0.8 mm. long, smooth; the peristome double, the teeth of the exostome long, narrowly lanceolate, perforated, smooth, reddish, the endostome rudimentary; operculum conic-rostrate, the beak short; spores round, averaging 40 μ, smooth; calyptra cucullate, pilose.

Type locality: Cuba, Oriente.

General distribution: West Indies and South America.

Illustrations: Sull., U. S. Expl. Exped. *T*, 10, 20; Broth., in E. & P. Nat. Pflanzenf. ed. 2, *f.* 546, *A-E*; Plate 8.

West Indies. CUBA: Oriente (in sylva Canelos), *H. Hioram* (NY). Oriente, *Sullivant* (the type of *Neckera crinita*), *Type* (PC). DOMINICAN REPUBLIC: Laudat, July 1, 1903, *Francis E. Lloyd* 280 (NY, FH). MARTINIQUE: On branches of trees (as *Meteorium crinitum*), 900 m. alt., 1899, *P. Duss* 189 (NY). GUADELOUPE: Hanging on branches, 1120 m. alt., 1898, *P. Duss* 340822 (NY). TRINIDAD: Morne Bleue, 1900 ft. alt., Dec. 1929, *P. W. Richards* 920 (NY).

The species is easily distinguished from *O. tetragona* by its plane branch leaves ending in long flexuose, filiform points as long as the rest of the leaf. So few collections of *O. crinita* have been made in the area covered by this study, that a discussion of its variability cannot be given here. For a comparison of *O. crinita* and *O. tetragona,* see comments under the latter species.

5. PTEROBRYOPSIS Fleischer, Hedwigia 45:56. 1906

Neckera Hook., Musc. Exot. Tab. 151. 1818-20, in part. *Meteorium*, Sec. *Esenbeckia* Mitt., Linn. Soc. London, Journ. Bot. 1:84. 1859. *Endotrichum* Jaeg., in Jaeg. & Sauerb. Ber. St. Gall. Nat. Ges. 1876-77:463 1878. *in part*. *Garovaglia* Paris, Index Bryol. ed. 2. 1:253. 1904. *in part*.

Plants medium size to robust, glossy, yellow-green to green, growing in lax colonies or tufts on trees; primary stems long, creeping, filiform, usually naked, leaves when present scale-like, lanceolate, to 0.5 mm. long; secondary stems branched, pinnate, often erect and stipitate, tumid, densely foliate; leaves crowded on the stem, appressed, weakly ranked, deeply concave, ovate from a subcordate base, short pointed, the margins entire or serrulate above, costate, rarely ecostate, apex acute, cells linear, smooth, porose, incrassate, alar cells very well defined (rarely inconspicuous), numerous, often quadrate, colored.

Dioicous. Perichaetial leaves narrowly pointed from a wide sheathing base. Sporophytes not seen.

Gemmae infrequent, cylindrical, uniseriate, borne in leaf axils.

Type species—Neckera crassicaulis C.M.

The large genus *Pterobryopsis* is represented by approximately forty-four species, principally of paleotropic distribution. In our area *Pterobryopsis* might be confused with *Renauldia* C.M., since both are similar in habit and general aspect, being laxly tufted and with turgid stems and leaves. The leaf in the latter genus however, is ecostate or very shortly bicostate and has inconspicuous alar cells. In the American species of *Pterobryopsis*, the single costa extending to beyond mid-leaf and the numerous rows of quadrate alar cells render the genus easy of recognition. There is only one species of this genus in the area covered by this study.

PTEROBRYOPSIS MEXICANA (Ren. & Card.) Fleischer, Hedwigia 45:60. 1906

Garovaglia mexicana Ren. & Card., Soc. Roy. de Bot. de Belg. Bul. 38(1):18. 1899. *Cryptotheca mexicana* Schimp. in Hb. *Pterobryopsis subfrondosa* Fleischer, Hedwigia 45:60. 1906. *nomen nudum*. *Pilotrichum subfrondosum* C.M. in Hb. *Pterobryopsis pringlei* Card., Rev. Bryol. 37:8. 1910.

Plants robust, rigid, yellow-green, slightly glossy, forming lax tufts or mats on trees and rocks; primary stems creeping, filiform, usually naked, leaves if present, minute, appressed, lanceolate, 0.4-0.5 mm. long, ecostate; secondary stems erect and stipitate to pendent, 6-8 cm. high, irregularly and loosely pinnately branched above; branches tumid, subjulaceous, densely foliate; leaves crowded, laxly imbricated, weakly ranked, deeply concave, often to 5 mm. long, to 2 mm. broad on the main stem, the branch leaves usually smaller, 1.5-3 mm. long, 1-1.5 mm. broad below, all leaves ovate, short acuminate, denticulate above, the apex acute; costa single, slender, ending at mid-leaf or a little above; cells long linear in outline, the walls more or less thickened, smooth, porose throughout, except for the alar group, median cells 28-64 μ x 4-10 μ, the cells of the apical region shorter, 14-48 x 4-10 μ, the cells of the insertion yellow, the alar group well defined, composed of numerous, quadrate, incrassate cells forming 8-20 rows, 9-24 μ x 9-22 μ.

Gemmae small, cylindrical, uniseriate, 3-12 cells long, borne in the axils of the leaves. Sporophyte unknown.

Type locality: Mexico, Herb. Röll.

General distribution: Mexico, Guatemala, Panama, and Costa Rica.

Illustrations: Bart., Mosses of Guatemala, *f.* 113, *A-C;* Plates 9, 10.

Mexico. VERACRUZ, Cordoba, ad truncos arborum in sylvis (the type of *Pilotrichum* (*Garovaglia*) *subfrondosum* C.M.) Sept. 1851, *C. Mohr* (NY).

Central America. GUATEMALA: Chimaltenango, along Rio Guascalate, southeast of Chimaltenango (on tree) 1700 m. alt., Dec. 14-23, 1940, *P. C. Standley* 81074a (F, BART, MICH). NICARAGUA: Jinotega, along trail between Jinotega and Las Mesitas, west of Jinotega (on tree trunk) 1000-1400 m. alt., June 21, 1947, *P. C. Standley* 9731 (F). COSTA RICA: Vicinity of San Jose, Santa Maria de Dota, 1600 m. alt., Dec. 1925, *P. C. Standley* 43252 (NY, FH). PANAMA: Chiriquí, Boquete, trail to Moenicke's, 3600-5000 ft. alt., *R. D. Svihla* 2722 (MICH).

The species shows a considerable degree of variation in aspect and in the relative size of its parts, but it is always identified at once by the rigid, concave, closely imbricated leaves with a single short costa and the numerous rows of quadrate alar cells. *Renauldia mexicana* (Mitt.) Crum, with which it may be superficially confused, differs significantly in having ecostate or shortly bicostate leaves, and a very inconspicuous alar region.

Cardot (1910) described *Pterobryopsis pringlei* from Mexico, as distinct from *P. mexicana*, and at that time noted the close relationships between the two species. He followed his original description with the comment, "Voisine du *P. mexicana* (Schimp.) Fleisch., cette espèce nouvelle s'en distingue par sa tiente verte, sa fronde plus allongée, ses rameaux non julacés, ses feuilles un peu comprimées, moins large et moins concaves, ovales-oblongues, et son tissu chlorophylleux."

When the specimens of *P. mexicana* and those of *P. pringlei* are compared, some variability in the flatness of the branches, the form of the leaf, the length of the cells and the thickening of their walls, and the density of the chlorophyll is noticeable in both. Furthermore, this variability overlaps from one to the other. *P. pringlei,* therefore, seems to be no more than a growth form of *P. mexicana,* with leaves less concave, and spreading rather than closely appressed, and the branching more irregular. Consequently it is here placed in synonymy with *P. mexicana.*

6. PTEROBRYON Hornschuch, in Martius' Fl. Bras. 1:50. 1840

Pterogonium Schwaegr., Suppl. III. I. 2, Tab. 243b. 1828. *Pilotrichum* Sect. VII. Pterobryum C.M., Syn. 2:179. 1851. in part. *Prionodon* Herzog, Bot. Centralbl. Beihefte Abt. B. 26(1):77. 1910.

Plants robust (rarely small), glossy, green or yellow-green, frondose or plumose, growing on bark or rocks; primary stems creeping, filiform, usually naked, leaves when present minute, appressed; secondary stems thickly and regularly pinnate above, with a woody stipe below, sometimes scraggly and ending in long flagelliform branches; leaves of secondary stems and branches rarely seriate, crowded, erect spreading, ovate-lanceolate or narrowly lanceolate, plicate to plane, seldom concave, often toothed at apex; leaves of the stipe similar, appressed or spreading, sometimes larger, broadly ovate to ovate-lanceolate; costa single in all leaves, strong, ending well above the middle, or percurrent; cells linear in outline, incrassate or thin-walled, smooth or rarely bearing a minute papilla at each end wall, the alar region scarcely differentiated, the basal cells yellowish or hyaline, often porose.

Dioicous. Male and female inflorescences borne on short lateral branches; perichaetial leaves oblong-lanceolate, costate; perigonia minute, the leaves ovate-lanceolate, ecostate; capsules immersed, ovoid, stomata none; peristome double, the exostome rudimentary, adherent to the teeth; the operculum short, conical; spores polymorphous, rounded to angular, smooth; calyptra small, mitriform, naked.

Gemmae when present, borne in axils of leaves, long cylindrical, hyaline, and uniseriate.

Type species—Pterogonium densum Schwaegr.

Hornschuch (1840) in Martius' *Flora Brasiliensis* created *Pterobryon,* the type genus of the Pterobryaceae, to accommodate the very distinctive *Pterogonium densum* of Schwaegrichen. For over a period of 110 years, however, his genus has been incorrectly spelled and interpreted as *Pterobryum* by botanists all over the world. This situation is understandable in part because generic names terminating in *-bryum* (*Rhodobryum, Müllerobryum, Anomobryum, Bryum,* etc.) are relatively frequent in the Bryophyta, and in speaking, the suffixes *-bryum* and *-bryon* are barely distinguishable. The name having thus taken hold remained a part of bryological literature until very recently.

Briquet (1935), in the *International Rules of Botanical Nomenclature,* Section 13, Art. 70, in dealing with the orthography of names states: "The original spelling of a name or epithet must be retained, except in the case of a typographic error, or of a clearly unintentional orthographic error. When the difference between two generic names lies in the termination, these names must be regarded as distinct, even though differing by one letter only. This does not apply to mere orthographic variants of the same name." Following these comments, a list of examples is given to illustrate the retention of original spellings in certain genera, one being comparable to the situation in *Pterobyron, . . . "Phoradendron* Nutt. must not be altered to *Phoradendrum."* Since Hornschuch (1840) used *Pterobryon* several times in his

original descriptions and in the index of volume I, part II, of the *Flora Brasiliensis* it is unlikely that his name was a typographic error. In following the rules of botanical nomenclature stated above, I see no alternative but to return to the original spelling of the genus as proposed by Hornschuch.

Pterobyron, meaning feather or wing moss, from the Greek, *ptero,* feather or wing, and *bryon,* tree moss, is appropriate for this group of large plumose, or fern-like mosses. The genus is represented in this area by *P. densum,* which is easily distinguished from any other member of the Pterobryaceae in the region. It is easy to recognize because of the very robust, dendroid, glossy green plants which resemble a tiny *Lycopodium.*

PTEROBRYON DENSUM (Schwaegr.) Hornschuh, in Martius' Fl. Bros. 1:51. 1840

Pterogonium densum Schwaegr., Suppl. III, I, 2. Tab. 243b. 1828. *Cryptotheca densa* Hornsch. in Deppe et Schiede Musc. Mexic. [Exsiccan]. *Cryptotheca densa* (Hedw.) Hornsch., ex Schlectendal & Chamisso, Linnaea 6:62. 1831. *Pilotrichum hornschuchii* C.M., Syn. 2:179. 1851. *Prionodon splendens* Herzog, Bot. Centralbl. Beihefte Abt. B. 26(1):77. 1910. *Pterobryum densum* var. *peracuminatum* Card., Rev. Bryol. 37:7. 1910. *Pterobryum panamense* Thér., Bull. Acad. Internat. Geog. Bot. 21:270. 1911. *Pterobryum densum* var. *condensatum* Lor. in Hb.

Schwaegrichen's original material was not available for study. His original description (1828) follows:

"Caulis primarius repens, rami triunciales, apice aliquot ramos vagos exserentes in Swartziano specimine, erecti, basi aphylli, apice ramis simpliciusculis confertis alternis, in comam subcompressam dispositis in caeteris speciminibus. Folia sparsa, erectiuscula, lanceolata acuminata in Swartziano), concava, grosse serrata, plicis binis versus apicem evanescentibus, pellucida, luteo-virentia; nervo tenui flavescente, versus apicem evanescente (num in Swartzianis ad apicem usque perduret, Swartzius non dixit, neque ipse efficere possum, ob factos omnium apices): cellulis oblongis, basiliaribus subquadratis. Flores probabiliter dioeci; masculi desunt. Feminei in rami oblongi, mox subulati, receptaculo crasso, carnoso; folia ovato-acuminata, striis expertia, nervo ad apicem evanescente, interiora capsulam superantia. Pistilla duodecim, filiformia, stylo turgido, fusca. Paraphyses nullae. Vaginula teres, e membrana valde crassa, luteo-badia. Pedunculus capsulam dimidiam longus erectus, badius. Capsula ovata, laevis, aequalis, badia. Peristomium basi planum, dein contractum in exiguum conum, capsulae orificium adnati, lanceolato-linares, simplici diametrum dimidium longum album; dentes intus, infra capsulae orificium adnati, lanceolato-lineares, simplici cellularum serie conflati, turgidi, albi. Membrana interna capsulae forma, sed brevitor. Columella oblonga, crassa, capsula parum tenuior, solida, mucrone tenui, inter dentes peristomii elevato, viridi-alba. Semina fusca. Operculum capsula duplo brevius, conico-restratum rectum, crassum ut peristomii dentes comprimat, capsulae concolor. Calyptra orperculo vix longior, campanulato-subulata, basiter fissa, rigida, lutescens, apice obscurius colorata."

The following description was drawn from material collected since that time:

Plants very robust, 4-10 cm. high, plumose, dendroid, glossy, green to golden green plants growing in thick erect tufts on trees; primary stems creeping, very slender, often naked, leaves when present minute, closely appressed, lanceolate, ecostate, to 0.5 mm. long; secondary stems dendroid, pinnately branched, the branches numerous or few, widely spreading, often forming a triangular frond; the leaves crowded, closely imbricated to erect spreading, not seriate on the stem, concave to flattened, strongly plicate, 3-5 mm. long, 0.8-2 mm. wide, ovate-lanceolate, apex acute to acuminate, serrate in upper half; costa single, slender, vanishing in the apex; cells linear-rhomboidal in outline, the walls more or less thickened, smooth, porose throughout, median cells 34-65 μ x 4-8 μ, the cells of the apical region often shorter, rhomboidal in outline, 14-40 μ x 4-12 μ, basal cells yellow, often porose, incrassate, 35-40 μ x 4-14 μ, the alar region poorly defined, the cells

small, 14-30 μ x 10-14 μ, subquadrate to irregular in outline, porose, yellow; stipe leaves appressed, scale-like, smaller than the branch leaves, 2-3 mm. long, 1 mm. wide, lanceolate, plane; costa weak, short, ending at mid-leaf, areolation as in the branch leaves.

Dioicous. Male and female inflorescences borne on short lateral branches; perichaetia to 5 mm. long, the leaves, large, concave, 3.5-5 mm. long, narrowly lanceolate, plane; costa narrow, ending in the apex, or ecostate, entire; areolation as in the ordinary leaves; perigonia very small, 0.8-1.5 mm. long, the leaves 1-15 mm. long, lanceolate, apex acute to short lanceolate, entire, ecostate, the areolation as in the branch leaves, antheridia 4-8; capsules immersed, oval-oblong, 2 mm. long, 1-1.5 mm. wide, light brown; seta very short, 0.5 mm. long; operculum conic-rostrate, the beak short; peristome double, the 16 existome teeth yellowish, narrowly lanceolate, smooth, cross striae present, often indistinct, the inner peristome rudimentary, fugacious; spores polymorphous, oval to oblong, sometimes angled, 60 x 32 μ, smooth, yellow; calyptra small, mitriform, smooth.

Gemmae borne on the stem in the axils of leaves, cylindrical, uniseriate, 3-12 cells long, the last 2 or 3 cells at either end often papillose.

Type locality: Brazil, in cortice arborum. *Sellow,* 4.

General distribution: Mexico, Central America, and South America.

Illustrations: Bart., Mosses of Guatemala, *f.* 116, *E-G;* Schwaegr., Suppl. III, *Tab.* 243, *f.* 1-15, 1828; Plates 11, 12, 13.

Mexico. VERACRUZ (on trunks of large trees in a dense forest near Jalapa), 4000 ft. alt., June 28, 1908, *G. C. Pringle* 10479 (NY, MICH, F, Y, MO, US, UC).

Central America. GUATEMALA: Suchitepequez, Volcan Santa Clara, between Finca El Naranjo and upper slopes (epiphyte on tree) 1250-2650 m. alt., May 23, 1942, *J. A. Steyermark* 46661 (NY, MICH, US, UC, TENN), 46663a (F). COSTA RICA: Limón, La Ascension, Rio Banano, 140-150 m. alt., June 1936, *F. Guitierrez,* 188 (NY, F). PANAMA: Chiriquí, above El Boquete, upper Caldera River near "Camp I," Holcomb's trail (on tree trunk in humid forest), 1450-1650 m. alt., Mar. 22-24, 1911, *W. R. Maxon* 5701 (NY, F, US, Y, FH, MICH).

BRAZIL: *Sellow,* 4: Type not seen.

P. densum is distinctive in its very robust, plumose, dendroid habit, its long, generally narrow, deeply plicate leaves which are toothed above, and in its immersed capsules. It sometimes reaches ten cm. in height, making it the largest representative of our frondose Pterobryaceae.

Some variation occurs in the leaf shape, length of acumen, and concavity of the leaves. The variability of this latter character was the basis for Cardot and Thériot's *P. panamense.* The original description of *P. panamense* says merely, "*A P. denso* foliis valde concavis jam diversum." The original collection shows intergradations of leaf shape from very concave to slightly concave forms, from narrowly lanceolate to ovate-lanceolate outline, and from acute to long acuminate apices. The very concave, acute leaves, are not as conspicuously plicate as the others, but both kinds may appear on the same stem. It would seem that their species was not based on constant characters.

Cardot, in 1910, described *P. densum* var. *peracuminatum* to include those plants having, "A forma typica foliis magis sensim angustatis, acumine longiore et angustiore distinctum." In his original material, the length of the acumen of the leaf varied from leaf to leaf, and it therefore should not be used as a reliable character in defining a variety.

The material of Lorentz' *P. densum* var. *condensatum* in the Herbarium of the New York Botanical Gardens, and at Paris, shows a negligible degree of variation in size and aspect of adult plants. Actually, this collection is composed of a few juvenile plants, and a dozen mature ones up to eight cm. in length. The species and varieties just mentioned are herein treated as synonyms of *P. densum.* Considering the specimens of this plant in the aggregate, a few with a lax habit, long flexuous branches, and rather distant,

narrow, acuminate leaves may always be found, but there appear to be no differences in sporophyte characters, and from the material examined all are evidently one specific unit.

7. PIREELLA Cardot, Rev. Bryol. 40:17. 1913

Neckera Hedw., Sp. Musc. 204. 1801. in part. *Pilotrichum* Palis. de Beauv., Prodr. 83. 1805. in part. *Pterobryun* Sect. II. *Orthostichella* Mitt., Linn. Soc. London, Journ. Bot. 12:426. 1869. *Pilotrichella* Jaeg., in Jaeg. & Sauerb. Ber. St. Gall. Nat. Ges. 1876-77:463. 1878. in part. *Pirea* Card., Soc. Roy. de Bot. de Belg. Bul. 32(1):175. 1893, non Durand, Ind. Gen. Phanerog. 494. 1888.

Plants medium size to robust (rarely very small), dull or glossy, green to yellow-green, dendroid, rarely loosely tufted, on decaying wood, bark, twigs, and rocks; primary stems creeping, filiform, usually naked, leaves when present minute, appressed, lanceolate; secondary stems often regularly pinnate above from a basal stipe, rarely simple, frequently ending in long, slender, flagelliform branches bearing minute, scale-like leaves; branch leaves crowded, clearly 4-5 seriate, erect or spreading, concave, broadly ovate to ovate-lanceolate (ligulate in *P. falcifolia*), rarely plicate, denticulate or entire, those of the secondary stems similar, somewhat larger; leaves of the stipe often different from the branch leaves, squarrose or appressed, larger (rarely smaller), broadly ovate to lanceolate, deeply concave, plane, denticulate or entire; costa single, or rarely short and double, stout or weak, percurrent, or ending at mid-leaf; cells linear in outline, thin-walled, or short-rhomboidal and incrassate, smooth or finely papillose at the ends, seldom porose throughout the whole leaf, the alar regions clearly defined or inconspicuous, yellow, the basal cells short, frequently porose, yellow.

Dioicous. Male and female inflorescences on short lateral branches; perichaetia large or small, leaves small, erect appressed, ecostate or costate, narrowly acuminate; perigonia small, the leaves minute, ovate-lanceolate, generally ecostate; capsules immersed or exserted, subglobose to oblong-oval, stomata none; seta long or very short, erect, smooth or scabrous, reddish; peristome double, the teeth of the exostome single or united in pairs, lanceolate, smooth, hyaline or reddish, endostome rudimentary; operculum conic-rostrate, the beak long; spores polymorphous, rounded, oblong, or angular, smooth; calyptra cuculate, smooth or pilose.

Gemmae long, septate, uniseriate, in the axils of the leaves.

Type species—Pirea mariae Cardot.

Cardot, in 1893, established the genus *Pirea* in memory of the well known Belgian botanist, Professor Louis Pire, and included in it the Costa Rican *P. mariae*. Later (1913), however, he realized that *Pirea* was the generic name given to a group of central Asiatic Cruciferae in 1888 by T. Durand in the *Index Generum Phanerogamorum*, and changed the name of his genus *Pirea* to *Pireella*.

Brotherus (1906), in his interpretations of the genus, subdivided the group into two sections:

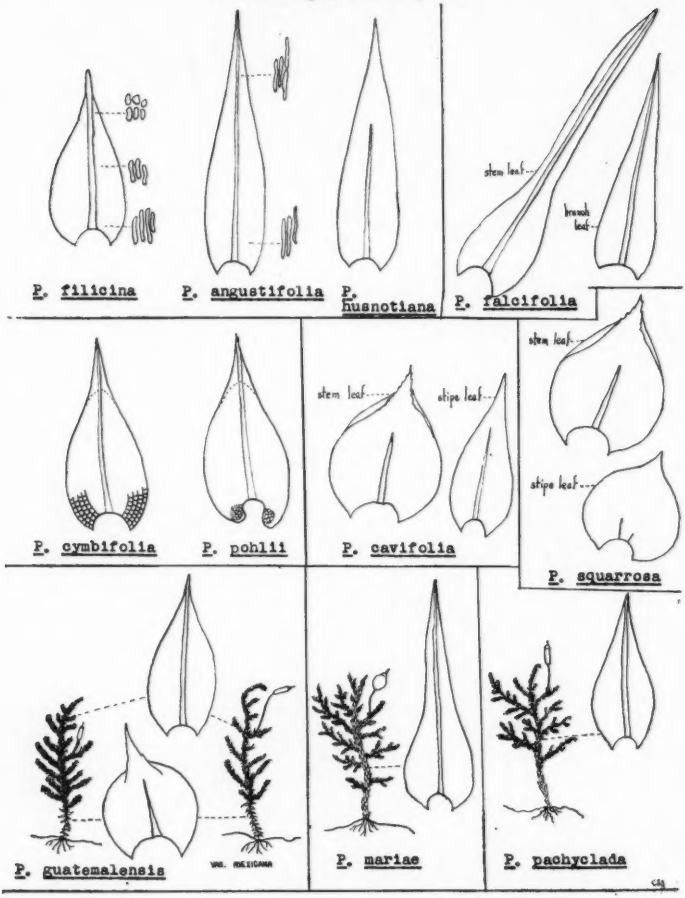
I. *Eu-Pireella*, with the cells of the lamina more or less thickened, the lumen oval, elongate or rhomboidal, the cells of the base near the costa linear in outline, those of the alar region quadrate in many rows, and the seta elongate.

II. *Pseudo-Orthostichella*, to include those species with the cells of the lamina mostly not thick-walled, the lumen elongate-elliptical or linear, those of the alar region not or scarcely differentiated; capsule immersed or emergent.

This treatment represents a purely artificial segregation in which several of the species might easily be included in both sections, and is not suitable as a classification for the American species.

Pireella, with twelve species and varieties, constitutes the largest genus of Pterobryaceae in the area considered in this study. Although the species within *Pireella* exhibit some variability, the genus itself is distinct, and is readily known by its typically frondose habit, long stipe, five seriate,

CHART 1.—Diagrams exaggerating important diagnostic characteristics of *Pireella* species. Leaves, ×18



broadly ovate to lanceolate, concave, leaves. Flagelliform branches and long, septate, uniseriate, gemmae borne in the axils of the leaves appear to be characteristic of this group, and have been observed in six of the species.

KEY TO THE SPECIES OF PIREELLA

1. Branch leaves falcate-secund, ligulate from an ovate base ..... *P. falcifolia*
1. Branch leaves erect spreading, ovate to narrowly lanceolate 2
2. Stipe leaves squarrose 3
2. Stipe leaves appressed 8
3. Branch leaves narrowly lanceolate, composed of long, linear, thin-walled cells 4

3. Branch leaves ovate to ovate-lanceolate, cells short, at least above, often incrassate .. 5
4. Costa percurrent ..... *P. angustifolia*
4. Costa ending at mid-leaf or a little above ..... *P. husnotiana*
5. Costa stout, percurrent; cells narrow and linear in the lower part of the leaf, becoming shorter and incrassate at the apex ..... *P. filicina*
5. Costa slender, ending at mid-leaf or just below the apex; cells short and incrassate throughout 6
6. Branch leaves broadly ovate, apiculate or ending in a short, acute apex ..*P. squarrosa*
6. Branch leaves ovate-lanceolate, acuminate 7
7. Seta 8-12 mm. long, smooth ..... *P. guatemalensis* var. *mexicana*
7. Seta 5 mm. long, scabrous above ..... *P. guatemalensis* var. *guatemalensis*
8. Cells short, often incrassate 9
8. Cells long, linear, thin-walled 11
9. Branch leaves broadly ovate, apiculate or ending in a very short, acute apex ..... *P. cavifolia*
9. Branch leaves ovate-lanceolate, acuminate 10
10. Secondary stem leaves twice the size of the branch leaves; capsules subglobose ..... *P. mariae*
10. Secondary stem leaves and branch leaves approximately the same size; capsules oblong-cylindrical ..... *P. pachyclada*
11. Alar region conspicuous, the cells numerous, quadrate, forming 6-16 rows up margin; basal angles of leaf abruptly cut off, never auriculate ..... *P. cymbifolia*
11. Alar region inconspicuous, the cells few, subquadrate, in small groups; basal angles of leaf rounded to auriculate ..... *P. pohlii*

PIREELLA MARIAE (Card.) Card., Rev. Bryol. 40:18. 1913

Pirea mariae Card., Soc. Roy. de Bot. de Belg. Bul. 32:176. 1893.

Plants medium size to robust, dendroid, dull green, growing in erect scraggly tufts on trees; primary stems stoloniferous, creeping, slender, naked; secondary stems 2-5 cm. high, erect, reddish, the stipe woody, 5 mm.-3 cm. long, loosely pinnately or bipinnately branched above; branch leaves crowded, imbricated, seldom erect spreading, disposed in five series, deeply concave, plane, 0.8-1.2 mm. long, 0.5-0.9 mm. wide, ovate-lanceolate, short acuminate, entire or minutely serrulate above; costa single, slender, vanishing just below the apex, rarely percurrent; secondary stem leaves similar to those of the branches but larger, 2-2.8 mm. long, 0.8-1.2 mm. wide below, ovate-lanceolate from a subcordate base, slenderly acuminate, entire; stipe leaves 0.5-0.9 mm. long, 0.3-0.6 mm. wide, closely appressed, ovate, short acuminate, entire, ecostate; upper and median cells of all leaves very short and oval in outline, or often quadrate, incrassate, smooth, linear near costa at base, median cells 5-8 μ x 4-8 μ, the cells of the apical region the same size, rarely to 16 μ long, cells of the lower third of the leaf adjacent to the costa 14-38 μ x 3-6 μ, those at the insertion yellow, seldom porose, 18-30 μ x 6-12 μ, the alar group poorly defined, the subquadrate cells few, yellow, 8-14 μ x 6-12 μ.

Dioicous. Male and female inflorescences borne on short lateral branches; perichaetia conspicuous, the leaves 1-3.2 mm. long, oblong-lanceolate, gradually narrowed to a long, slender, denticulate point, or ovate and short acuminate, costa single, ending in the long acumen, areolation as in the ordinary leaves; perigonia small, to 1.5 mm. long, the leaves ovate, ecostate, entire; capsules exserted, erect or slightly inclined, subglobose, 2 mm. long, 1.7 mm. wide, the mouth small; seta slender, 6-10 mm. long, scabrous above, reddish; operculum conic-rostrate, 1.5 mm. long; peristome double, the teeth of the exostome lanceolate, irregular, smooth, hyaline, cleft along median line, 0.18 mm. long, rarely united above, the endostome rudimentary, fugacious; spores polymorphous, round, oblong, or angled, smooth, 22-48 μ.

Type locality: Costa Rica, forêts de Juan Viñas, *H. Pittier*.

General distribution: Mexico and Central America.

Illustrations: Bart., Mosses of Guatemala *f.* 115, A-D; Plate 24.

Mexico. PUEBLA, El Cerro de Cuhautepec (on bark), 2200 ft. alt., Nov. 22, 1944, *A. J. Sharp* 1335a (TENN), 2800 ft. alt., Nov. 23, 1944, *A. J. Sharp* 1351 (TENN).

Central America. GUATEMALA: Izabal, Cerro San Gil (epiphyte in damp, forested slopes and barrancas), 330-900 m. alt., Dec. 25, 1941, *J. A. Steyermark* 41890 (F, UC,

BART, MICH). COSTA RICA: Forêts de Juan Viñas, 1135 m. alt., Jan. 25, 1890, *H. Pittier* 5653, *Type* (PC). PANAMA: Chiriquí, Boquete (on tree), Aug. 1950, *A. M. Chickering* (MICH).

P. mariae differs from all other species in the genus in that the secondary stem and branch leaves are not alike in size and shape, the alar region is inconspicuous in all leaves, the stipe leaves are appressed, the setae are scabrous above, and the capsules are sub-globose (see chart 1). This infrequent species often bears abundant fruit, and shows little variability in contrast to most of the other species.

PIREELLA PACHYCLADA (Ren. & Card.) Card., Rev. Bryol. 40:18. 1913

Pirea pachyclada Ren. & Card., Soc. Roy. de Bot. de Belg Bul.. 41(1):67. 1905. *Pireella cubensis* Card. & Thér., Rev. Bryol. 40:17. 1913.

Plants medium size, dendroid, dark green to golden green, seldom glossy, growing in erect tufts on trees and rocks; primary stems stoloniferous, creeping, slender, usually naked, leaves when present minute, 0.5 mm. long, distant, lanceolate, ecostate; secondary stems 1.5-6 cm. long, erect, reddish, dendroid, pinnately branched; the branches short, to 1.8 cm. long, the woody stipe 4.5-12 mm. long; branch leaves closely imbricated, ranked in five distinct series, deeply concave, plane, 1.8-2.5 mm. long, 1.5-1.8 mm. wide, oblong-ovate from a cordate base, short acuminate, margins serrulate above or entire; costa single, slender, vanishing in the apex; secondary stem and stipe leaves similar to the branch leaves often to 3 mm. long, the stipe leaves appressed; cells of all leaves predominantly short, oval and incrassate, smooth, porose only at the insertion; median cells oval-rhomboidal in outline, 8-9.6 μ x 4-5 μ, those adjacent to the costa at mid-leaf long, linear, 25-40 μ x 3-5 μ, sinuous, the cells of the apical region sometimes long, 6-24 μ x 3-9 μ, basal cells 28-33 μ x 10-14 μ, porose, yellow, the alar group very conspicuous, composed of 4-10 rows of small, quadrate, cells 7-9 μ x 6-9 μ.

Dioicous. Male and female inflorescences borne on short lateral branches; the perichaetia conspicuous, to 3 mm. long, the leaves 2.5-3 mm. long, ovate to oblong-lanceolate, abruptly narrowed into a long, serrulate, hair-like point; costa single, ending in the long acumen, rarely absent, cells longer and narrower throughout than in the ordinary leaves, 25-40 μ x 3-5μ; perigonia very small, to 2 mm. long, the leaves ovate, ecostate, entire; capsules exserted, oblong-cylindric, 2-2.5 mm. long, 0.35 mm. wide, brown, narrowed at the mouth; seta erect, 4-5 mm. long, reddish, smooth, flexuous; peristome not seen.

Type locality: Mexico: Yucatan, San-Geronimo.

General distribution: Mexico and Central America.

Illustrations: Ther., 1940, Mem. Soc. Cub. Hist. Nat. 14(4):*Pl.* 54, *f.* 6, *a-f;* Bart., Mosses of Guatemala, *f.* 114;*G-J;* Plates 16, 17.

Mexico. YUCATAN, San-Geronimo, 1847, *Morelet, Type* (PC).

Central America. GUATEMALA: Petén, Vaxactum, Apr. 5, 1931, *H. H. Bartlett* 12486 (NY, US, FH, MICH, BART).

West Indies. CUBA: Pinar del Rio, San Diego de los Banos (the type of *P. cubensis*), Oct. 10, 1905, *Van Hermann* 3182 (PC).

The distinguishing characteristics of the species are the appressed stipe leaves, the similarity in size and structure of the secondary stem and branch leaves, the short oval outline of the median and upper leaf cells, and the numerous rows of quadrate alar cells in all leaves. *P. mariae* is closely allied to *P. pachyclada* but differs in that in the former species the stem and branch leaves are not similar in size and shape, the area of alar cells is much smaller, the setae are scabrous above, and the capsules are subglobose.

The species, *P. pachyclada,* exhibits only a slight degree of variability in the size of plants, leaves, cells, and in alar region. Cardot and Thériot (1913), published *P. cubensis* which differed from *P. pachyclada* . . . "par ses rameaux moins épais et moins obtus, par ses feuilles raméales plus étroites,

avec les cellules alaires plus grandes et moins obscures." A comparison of the original collections of both species show that the above distinctions drawn from vegetative characters do not hold, for variations in the branching, size of leaves, and number or size of alar cells have been found indiscriminately in plants of both. All of these variations are well within the admitted limits of *P. pachyclada*, and in my estimation, there is no basis for separating *P. cubensis* from *P. pachyclada*.

PIREELA GUATEMALENSIS Bart. Bryol. 49:116. 1946

var. GUATEMALENSIS

Thamnium guatemalense Williams in Hb. *Pirea guatemalensis* Broth. in Hb.

Plants medium size to robust, dendroid, dull yellow-green, growing in long erect scraggly masses on trees, logs, and rocks; primary stems stoloniferous, creeping, slender, naked; secondary stems 1.8-6 cm. high, erect, reddish, densely pinnately or bipinnately branched, the branches to 2 cm. long, the stipe woody, 5 mm.-2.5 cm. long; branch leaves crowded, imbricated to erect-spreading, disposed in five series or weakly ranked, deeply concave, plane, ovate-lanceolate, acute to acuminate, to 1.3 mm. long, 0.9-1 mm. wide, basal angles rounded to auriculate, the margins entire or serrulate above; costa single, slender, vanishing just below the apex, rarely percurrent; secondary stem leaves like those of the branches but larger, broadly ovate, 2 mm. long, 1.2 mm. wide; stipe leaves very small, squarrose-recurved, 0.8-1 mm. long, 0.8 mm. wide below, triangular-ovate, abruptly acuminate, margins entire or serrulate, the costa single, ending just below the apex; cells of all leaves short and oval above the middle, becoming linear in the lower part, incrassate, smooth, seldom porose, median cells and those in the lower third of the leaves linear, sinuous, 17-46 μ x 3-6 μ, the cells of the apical region short, oval, 4.5- 16 μ x 4-12 μ, cells at the leaf insertion linear in outline, yellow, 8-16 μ x 4-14 μ, sometimes porose, the alar group poorly defined, the cells few, subquadrate to short rectangular, yellow, 8-14 μ x 6-10 μ.

Dioicous. Male and female inflorescences borne on short lateral branches; perichaetia conspicuous, the leaves 2-3 mm. long, oblong-lanceolate, gradually narrowed into a long linear, serrulate point, costa single, ending in the long acumen, areolation as in the ordinary leaves; perigonia small, 1.8 mm. long, the leaves ovate, ecostate and entire; capsules exserted, erect, oblong-ovate, 2 mm. long, 0.8 mm. wide, brown; seta erect, to 5 mm. long, scabrous above, smooth below; operculum erect, conic-rostrate.

Type locality: Guatemala, Alto Verapaz, Finca Mocca, *H. Johnson.*

General distribution: Guatemala.

Illustrations: Bart., Mosses of Guatemala, *f.* 115, *E-I;* Plate 21.

Central America. GUATEMALA: Alta Verapaz, Finca Mocca, *Harry Johnson* 154a, *Type* (F, BART).

The distinguishing characteristics of var. *guatemalensis* are the squarrose stipe leaves, the short setae (to 5 mm. long), and the oblong-ovate capsules. The important difference between var. *guatemalensis* and the new variety *mexicana* is the short seta which is scabrous above and smooth below in var. *guatemalensis*. The secondary stem leaves are often more markedly auricled than in var. *mexicana*, but this character is variable and not always reliable. *P. guatemalensis* var. *guatemalensis* is thus far known in fruit only from the original collection.

Pirella guatemalensis Bart. var. **mexicana** var. nov.

A priori differt: Seta erecta, 8-12 mm. longa, laevissima, flexuosa; theca erecta, elliptica, fusca, deoperculata circa 3 mm. longa, 0.5-0.7 mm. lata; operculum conico-rostratum, 1.5 mm. longum; peristomium simplex, dentes hyalinae, laeves, anguste lanceolati; spori polymorphae, laeves, virides.

This variety differs from var. *guatemalensis* as follows:

Dioicous. Perichaetia large, the leaves 2-3 mm. long, oblong-lanceolate, gradually

narrowed into a long linear, serrulate point, costa single, vanishing in the long acumen; capsules exserted, erect, oblong-cylindric, 3 mm. long, 0.5-0.7 mm. wide, brown; seta erect, reddish, flexuose, 8-12 mm. long, smooth or rarely finely scabrous above; operculum erect, conic-rostrate, 1.2 mm. long; peristome teeth lanceolate, smooth, yellow, perforated below, very irregular, 0.18-0.24 mm. long; spores polymorphous, angular to rounded, smooth, green, 22-38 μ.

Type locality: Mexico, Oaxaca, Choapan, region of Chinantla, Toabela, San Juan de Petlapa, *J. V. Santos.*

General distribution: Mexico.

Illustrations: Plate 21.

Mexico. OAXACA, Choapam, region of Chinantla, Toabela, San Juan de Petlapa, dense, sub-temperate forest on rocky mountain slopes (on rock), Oct. 26, 1944, *J. V. Santos* 3431, *Type* (BART, MICH).

From the var. *guatemalensis*, *P. guatemalensis* var. *mexicana* is distinct in the long smooth seta, and the narrowly oblong capsules to 3 mm. long. The secondary stem leaves of var. *mexicana* are often less conspicuously auriculate than those of var. *guatemalensis*, but final separation must rest on sporophyte features. The var. *mexicana* is easily distinguished from all other members of the genus by its squarrose stipe leaves and smooth setae which reach 12 mm. in length. It has appeared in collections with more frequency than *P. guatemalensis* var. *guatemalensis*.

PIREELLA FILICINA (Hedw.) Card., Rev. Bryol. 40:18. 1913

Neckera filicina Hedw., Sp. Musc. 204. 1801. *Pilotrichum filicinum* Palis. de Beauv., Prodr. 83. 1805. *Pterobryum filicinum* (Hedw.) Mitt., Linn. Soc. London, Journ. Bot. 12:425. 1869. *Pirea filicina* (Hedw.) Broth., in E. & P. Nat. Pflanzenf. ed. 1. 1(3):796. 1906. *Pireella panamensis* (Broth. & Paris ined., nomen) Card., Rev. Bryol. 40:18. 1913. *nomen nudum.*

Plants medium size, dendroid, dark green to yellow-green, usually dull, growing in erect tufts on trees, logs, and rocks; primary stems stoloniferous, creeping, filiform, naked, leaves when present, minute, 0.4-0.5 mm. long, distant, closely appressed, lanceolate, and ecostate; secondary stems 2-8 cm. long, erect, reddish, dendroid from a woody stipe 4-7 mm. long, thickly pinnately branched, the branches short, to 2 cm. long, bearing minute, lanceolate, scale-like, ecostate leaves, 0.5 mm. long, the flagelliform branches often "rooting" and producing ordinary leaves at their apices; branch leaves crowded, imbricated to erect-spreading, ranked in five distinct spiral rows, deeply concave, plane, 1.5-2.5 mm. long, 0.9-2 mm. wide, ovate-lanceolate, short acuminate, margins serrulate above, often entire; costa single, well developed, percurrent, often becoming flat and broader in the apical region; secondary stem leaves like the branch leaves, often larger, to 3 mm. long; stipe leaves squarrose to widely spreading, large, 1.8-3 mm. long, 2 mm. wide below, distant, plane, abruptly acuminate from a broad, short, cordate base, margins entire or serrulate; costa single, percurrent; cells of the branch and secondary stem leaves long, linear in outline, sinuous below, more or less thick-walled, short, and oval above, incrassate, seldom porose, often bearing a fine papilla on each end wall, the papillae dorsal and localized to the upper half of the leaves, rarely throughout their length; median cells 34-48 μ x 3-6 μ, the apical cells very short, oval to rhomboidal in outline, 4-10 μ x 2-9 μ, cells at the insertion short, 15-35 μ, yellow, porose, incrassate, the alar region poorly defined, the cells few, yellow, porose, irregular to subquadrate in outline, 15-24 μ x 4-8 μ, the walls more or less thickened; cells of stipe leaves as in branch and secondary stem leaves, often linear in outline, and sinuous throughout, but shorter along the insertion.

Dioicous. Male and female inflorescences borne on short lateral branches; perichaetia large, 6 mm. long, the leaves 3.5-6 mm. long, lanceolate, gradually narrowed to a long hair-like, serrulate or entire point, costa single, stout, ending in the long acumen, areolation as in ordinary leaves, cells often longer, to 75 μ long, to 3 μ wide; perigonia small, 1.5 mm. long, the leaves ovate, ecostate, entire; capsules immersed, oval-cylindric, brown, 2-2.25 mm. long, 0.5-1 mm. wide, narrowed at the mouth; seta 0.4-0.5 mm. long; operculum conic-rostrate, the beak to 1 mm. long, pointed; peristome double, the exostome teeth lanceolate, smooth, hyaline, often perforate at the base, very irregular, 0.18-0.20 mm.

long, endostome rudimentary, fugacious; spores round, smooth or finely papillose in part, 20-35 μ in diameter; calyptra cucullate, 2 mm. long, pilose.

Gemmae frequent, septate, 3-10 cells long, uniseriate, in the axils of stem leaves.

Type locality: Jamaica, near Coldspring, high mountains (on trunk of trees).

General distribution: West Indies.

Illustrations: Hedw., Sp. Musc. *pl.* 18; Plate 19.

West Indies. JAMAICA: Near Coldspring, high mountains (on trunks of trees), 1803, *Swartz, Type,* Ex. Herb. Hort. Bot. Reg. Kew (NY). CUBA: Near Santiago, Sevilla Estate, Ubero River, 2000 ft., alt., Sept. 18, 1906, *Norman Taylor* 267 (NY), Baracoa to Jiquarito Mt. (on rocks), 2400 ft., alt., Sept. 17, 1906, *Norman Taylor* 468b (NY). HAITI: Morne Chavary, vicinity of St. Louis du Nord (on tree trunk, summit southwest of city), Mar. 30-Apr. 7, 1929, *E. C. Leonard & G. M. Leonard* 14499 (NY, FH).

PANAMA: (the type of *P. panamensis*) 1900, *F. Hélion* (PC).

The distinguishing characteristics of this species are its squarrose stipe leaves, the ovate-lanceolate branch leaves with long, narrow cells in the lower part, and shorter, oval often thicker walled cells in the apical region, and the immersed capsules. *P. angustifolia,* to which it is closely allied differs in having long narrowly lanceolate leaves with long narrow cells throughout (see chart 1).

The original collection of *P. panamensis* at Paris, collected in Panama by Hélion (1900), is rather poor material and identical with that of *P. filicina.* The species exhibits little variation.

Pireella angustifolia (C.M.) comb. nov.

Pilotrichum angustifolium C.M., Syn. 2:181. 1851. *Pterobryum angustifolium* (C.M.) Mitt., Linn. Soc. London, Journ., Bot. 12:426. 1869. *Pterobryum angustifolium* forma *flagellifera* Besch., Ann. des Sci. Nat., Bot. VI. 3:201. 1876. *Pterobryum integrifolium* Hampe, in Besch., Journ. de Bot. 8:62. 1894. *Pterobryum angustifolium* var. *flabelliforme* Besch. in Hb.

Plants medium size to robust, dendroid, dark green or yellow-green, glossy, growing in erect tufts on trees, logs, and rocks; primary stems stoloniferous, creeping, slender, usually naked, leaves when present minute 0.4-0.5 mm. long, distant, closely appressed, lanceolate, and ecostate; secondary stems 2-7 cm. long, erect, red, dendroid, from a woody stipe, 0.5-2 cm. long, pinnately branched, the branches to 2 cm. long, flagelliform branches frequent, to 6 cm. long, often "rooting" and developing ordinary leaves at their apices, the leaves of the flagelliform branches, 0.4-0.9 mm. long, caducous, lanceolate, scale-like, ecostate; branch leaves crowded, imbricated to erect spreading, disposed in five series or weakly ranked, concave, plane or weakly plicate below, 1.4-3.5 mm. long, 0.8-1.3 mm. wide, narrowly lanceolate, acuminate, margins entire or serrulate above; costa single, slender, percurrent; secondary stem leaves like the branch leaves, longer, 3.5 mm. long; stipe leaves squarrose-recurved, large, 1.8-3.2 mm. long, 2.2 mm. wide below, distant, plane, triangular-ovate, abruptly acuminate, the margins entire or serrulate; costa single, percurrent; cells of all leaves long, narrowly linear in outline, often sinuous, thin-walled, seldom porose, rarely bearing a small fine papilla at either end; median cells 35-55 μ x 3-6 μ, the cells of the apical region similar or somewhat shorter, 28-45 μ x 4-8 μ, cells of the insertion short, 15-34 μ x 4-8 μ, yellow, porose, often incrassate, the alar group poorly defined, the cells few, yellow, porose, the walls more or less thickened, irregular to subquadrate, 15-25 μ x 4-8 μ.

Dioicous. Male and female inflorescences borne on short lateral branches; perichaetia conspicuous, to 5 mm. long, the leaves 3-5 mm. long, oblong-lanceolate, gradually narrowed to a long linear, denticulate point, the costa single, ending in the long acumen, areolation as in the ordinary leaves; perigonia small, to 2 mm. long, the leaves ovate, ecostate, entire; capsules immersed, erect, ovoid, 2-2.5 mm. long, 1 mm. wide, narrowed at the mouth; seta 0.4-0.5 mm. long; operculum conic-rostrate, 1.2 mm. long; peristome double, the exostome teeth lanceolate, smooth, yellow, sometimes united above, often perforate at base, very irregular, short, 0.18-0.22 mm. in length, endostome rudimentary, fugacious; spores round, smooth, 22-36 μ in diameter; calyptra cucullate, 2 mm. long, pilose.

Gemmae frequent, septate, 3-10 cells long, uniseriate, in axillary clusters on the stem.
Type locality: Trinidad, Monte Tocuchi, 3400 ft. alt., 1947, *Crüger.*
General distribution: Central America and West Indies.
Illustrations: Bart., Mosses of Guatemala, *f.* 117, *A-C;* Plate 18.

Central America. GUATEMALA: Izabal, between Virginia and Lago Izabal, Montaña del Mico (on bark of living tree) 50-500 m. alt., Apr. 5, 1940, *J. A. Steyermark* 38892 (NY, F, UC, BART, MICH). HONDURAS: Bonacca Island (on twigs in the mountains), *Gaumer* (no date), (NY, F). NICARAGUA: Oct. 12, 1900 (no data), *N. L. Britton* (NY). COSTA RICA: Guanacaste, Naranjos Agrios, 600-700 m. alt., Jan. 29, 1926, *P. C. Standley & J. Valerio* 46404 (NY, BART). PANAMA: Cerro Flores (on fallen log), 5500 ft. alt., Mar. 18, 1924, *J. M. Valentine* (NY, Y).

West Indies. JAMAICA: Mountain trail between House Hill and Cuna Cuna Gap, St. Thomas (on tree trunks) 550-725 m. alt., June 7, 1926, *W. R. Maxon* 8960 (NY). CUBA: Sti. Spiritus Mts., Sta. Clara, Sierra del Caballette Aug. 1-11, *Bros. Leon & Clement,* 6781 (NY). HAITI: Departement du Nord, vicinity of Marmelade (as *P. filicina*), 800 m. alt., Dec. 20, 1925, *E. C. Leonard* 8271 (NY). TRINIDAD: Antillarum Monte Tocuchi, 3400 ft. alt., 1847, *Crüger, Type* (NY). MARTINIQUE: Medaille to St. Joseph by Trace de Boutaud, 500 m. alt., Dec. 20, 1940, *H. Stehlé* 3782 (NY), between Colson and l'Alma, 550 m. alt., June 1920, *M. & H. Stehlé* 3935 (F), 2936 (NY). VIRGIN ISLANDS: Tortola, Sage Mountain, May 1913, *W. C. Fishlock* 85 (NY). GUADELOUPE: Morne Caraibe (endroit sec) Jan. 1907, *P. Duss* (NY). BARBADOES: (no data), (as *Pilotrichum filicinum*), *Parker* (NY). ST. VINCENTS: (no data), Mar. 1890, *H. H. & G. W. Smith* 1478 (NY, FH). GRENADA: Grand Etang near Rest House, Mar. 29, 1897, *W. E. Broadway* (NY, FH). DOMINICAN REPUBLIC: Soufriere, 1903, *F. E. Lloyd* 506 (NY).

The distinguishing characters of *P. angustifolia* are its squarrose, cordate stipe leaves, and long, narrowly lanceolate branch leaves. The percurrent costa of the leaf will serve to separate this species from the closely related *P. husnotiana,* which has a weak costa ending at mid-leaf or a little above. There has been considerable confusion in distinguishing between *P. angustifolia* and *P. filicina* (Hedw.) Card., largely because immersed capsules and squarrose stipe leaves are peculiar to both species. *P. filicina,* however, has conspicuously five-seriate, ovate-lanceolate branch leaves, with short oval cells in the apical region, while the branch leaves in *P. angustifolia* are weakly ranked, and have long, narrow cells throughout the leaf. For a comparison of these species see chart 1.

I have transferred this species, which for many years has been included under *Pterobryon,* to *Pireella.* Its distinguishing characteristics, the flagelliform branches, the five-seriate, lanceolate leaves, and the cells with a single papilla at either end on the dorsal side, are characteristic of the genus *Pireella* rather than of *Pterobryon;* furthermore, the species as already noted above has many characters in common with *Pireella filicina.* The immersed capsules of *P. angustifolia* and *P. filicina* are distinctive in this genus, since the capsule is exserted in most species, but the reduction of seta length is here regarded as representing one of the developmental lines within the group.

This species is made up of a number of minor variants, which are not of sufficient degree or stability to warrant separate classification. For this reason I have set up a number of basic criteria, with allowance for deviations in either direction, and have drawn the concept of the species from the entire group rather than from the single individual (the type specimen). The species seems to be an extremely plastic one and certain combinations of the variations, when in the extreme, make for individuals which, to one not familiar with the plants collected over a wide area, might be considered

separate and distinct species. Thus, Bescherelle's (1876) forma *flagellifera*, later raised to specific rank by Hampe (1894), as *Pterobryum integrifolium* included those plants bearing many flagelliferous branches. Whether the development of flagelliferous branches in *P. angustifolia*, or in any other species within the genus is strictly a response to an environmental situation or strictly a genetic variation must for the present, remain unknown. No decisive answer is possible at this time, for more material and a further knowledge of the substratum, environmental conditions, and nature of the plants are necessary in order to supply the needed critical information Although the fragmentary remnants of the original collection made by Crüger in Trinidad show no flagelliform branches, specimens from numerous localities bear them, and it is not uncommon to find among the several secondary stems arising from a primary stem one plant with a single short flagelliform branch. Therefore, the presence of flagelliform branches is no justification for a special formal taxon, but it should be considered an inherent characteristic well within the given limits of the genus *Pireella* and its species. Bescherelle noted the diversity of size and habit in this plant, and used the herbarium name, *P. angustifolium* var. *flabelliforme* for robust specimens with a fan or wedge-shaped branching pattern. In this genus size and habit alone are not very trustworthy characters on which to separate varieties and species.

PIREELLA HUSNOTIANA (Besch.) Card., Rev. Bryol. 40:18. 1913

Pterobryum husnotianum Besch., Ann. des Sci. Nat., Bot. VI. 3:201. 1876. *Pirea husnotiana* (Besch.) Broth., in E. & P. Nat., Pflanzenf. ed. 1. 1(3):796. 1906.

Plants medium size, dendroid, green, glossy, growing on trees; primary stems creeping, naked; secondary stems 2-5 cm long, dendroid, erect, reddish, pinnately branched, the branches to 1.5 cm. long, the stipe woody, 5 mm.-1.8 cm. long; branch leaves crowded, imbricated to erect spreading, weakly ranked or disposed in five series, concave, plane, 1.5-2.5 mm. long, 0.7-1 mm. wide, ovate-lanceolate to narrowly lanceolate, acuminate, margins entire or serrulate above; costa single, weak, ending at mid-leaf, or in the upper third; secondary stem leaves like the branch leaves, often longer, to 2.8 mm.; stipe leaves squarrose-recurved, large, 1.6-2.8 mm. long, 2 mm. wide below, distant, plane, triangular-ovate, abruptly acuminate, the margins entire or serrulate above; costa single, vanishing in the apex; cells of all the leaves long, narrowly linear in outline, often sinuous, thin-walled, seldom porose, smooth, median cells 28-48 μ x 3-7 μ, the cells of the apical region somewhat shorter, 24-42 μ x 3-8 μ, cells at the insertion shorter, 15-32 μ x 4-10 μ, yellow, porose, incrassate, the alar group poorly defined, the cells few, irregular to subquadrate in outline, 12-25 μ x 4-10 μ, yellow, porose, the walls more or less thickened.

Gemmae septate, 3-8 cells long, uniseriate, borne in the axils of the leaves.

Sporophyte not seen.

Type locality: Martinique, Pitons du Carbet; Guadeloupe, Le Houelmont, *Husnot*.

General distribution: West Indies.

Illustrations: Plate 18.

West Indies. MARTINIQUE, (sur les arbres des forêts), Pitons du Carbet; GUADELOUPE, Le Houelmont, 500-700 m. alt., 1868, *Husnot*, 171. *Type* (NY).

The distinguishing characters of the species are its squarrose stipe leaves, and secondary stem and branch leaves with long, narrow cells, and a costa ending just above the middle of the leaf. The broader, usually smaller leaves and shorter costa separate it from *P. angustifolia* to which it is closely related. *P. husnotiana* is known only from the original material collected in the West Indies.

Bescherelle (1876) described the perichaetial leaves as large, very long

acuminate, having the apices crenulate, and the costae long. The sporophyte is unknown.

PIREELLA CAVIFOLIA (Card.) Rev. Bryol. 40:17. 1913

Pirea cavifolia Card., Rev. Bryol. 37:39. 1910. *Jaegerinopsis* (?) *ramosa* Ther., Soc. Cubana de Hist. Nat. "Felipe Poey" Mem. 14(4):358. 1940.

Plants small to medium size, dendroid, green to yellow-green, glossy, growing in erect scraggly masses on trees; primary stems stoloniferous, creeping, slender, naked or leaves when present minute 0.4-0.5 mm. long, distant, closely appressed, lanceolate, ecostate; secondary stems 1.5-4 cm. long, erect, loosely pinnate, the branches to 1.3 cm. long, stipe woody, 5 mm.-1.5 cm. long; flagelliform branches sometimes present, caducous, filiform, delicate, to 1 cm. long, 48 μ wide, bearing minute scale-like leaves 118-128 μ x 46 μ in size; branch leaves crowded, imbricated to erect spreading, in five series, deeply concave, not plicate, 0.9-2.5 mm. long, 0.5-1 mm. wide, broadly ovate to ovate-lanceolate, apiculate or short acuminate, margins entire or serrulate above; costa single, slender, ending at mid-leaf or somewhat above; secondary stem leaves similar to those of the branches, sometimes larger, to 2.8 mm. long; stipe leaves closely appressed, few, otherwise like the stem and branch leaves; cells of all leaves linear, more or less thick-walled, often with a single, small papilla at either end on the dorsal side; median cells 32-54 μ x 5-8 μ, the cells of the apical region short, oval to rhomboidal in outline 8-17 μ x 4-8 μ, cells at the insertion 17-40 μ x 3-10 μ, yellow, the alar group poorly defined, the cells few, yellow, incrassate, irregular to subquadrate, 8-11 μ x 7-9 μ.

Sporophyte unknown.

Type locality: Mexico, Hidalgo, Honey Station, *C. G. Pringle.*

General distribution: Mexico and West Indies.

Illustrations: Ther., 1940. Mem. Soc. Cub. Hist. Nat. 14(4). *pl.* 54; Plates 22, 23.

Mexico. HIDALGO, Honey Station, 1909, *C. G. Pringle* 15668, in part. *Type* (PC, BART).

West Indies. JAMAICA: Blue Mountains, Jan. 1928, *C. R. Orcutt* (NY, BART). CUBA: Oriente, Sierra Maestra, in the dwarf forest near La Gran Piedra, 1100 m. alt., (on arborescent fern), Nov. 10, 1917, *E. L. Ekman* 8790 *in part* (the part of *Jaegerinopsis? ramosa*) (PC). PUERTO RICO: Barranquitas, upper slopes of La Torrecilla, Oct. 28, 1939, *W. C. Steere* 4657 (FH, MICH).

This is one of the most delicate species of the genus, and is distinct from all others in its broadly ovate, apiculate leaves, short costa ending in the upper third of the leaf, and appressed stipe leaves. *P. cavifolia* bears a close resemblance to *P. squarrosa* both in habit and areolation, but the latter plant differs conspicuously in having very small, squarrose, stipe leaves. The curious filiform flagelliform branches of *P. cavifolia* are different from those of the other species, in that they are very fragile and easily break up into minute fragments which suggest gemmae of a sort.

At the time Thériot (1940), published *Jaegerinopsis? ramosa* he expressed considerable doubt as to the identity of his plant, and added the following comments to the original description: "Il est difficile de classer cette mousse à cause de sa stérilité. Elle emprunte quelques caracteres, le port entre autres, au genre *Renauldia*, d'autres aux genres *Hildebrandtiella* et *Jaegerinopsis* comme la forme des feuilles, le tissu, la nervure; mais l'absence de certains caracteres empeche de l'attribuer, avec certitude, a l'un ou a l'autre de ces genres. Elle represent peut-etre un nouveau genre? . . . je la rattache au genre *Jaegerinopsis* avec un point de doute, puisque dans ce genre les tiges secondaires sont simples et les feuilles non involutées." An examination of the type material from the Museum at Paris clearly shows that his plants belong to the genus *Pireella*.

Pireella squarrosa sp. nov.

Caulis primarius filiformis, repens, secundarius erectus, ad 2 cm. altus, irregulariter dendroideus, stipite brevi, inferne simplex foliis squarrosis obtectus, superne in frondem pinnatam divisis; ramis confertis, brevis. Folia caulina superiora erecto-patentia, concava, late ovato-acuminata, 1.8-2.5 mm. longis, 1-1.5 mm. lata; margines ubique arcte serrulati, plani; costa tenuis, sub apice evanida; cellulae lineares, ad 25-50 μ x 3-6 μ, basilares breviores, hyalinae, alares pauces, subquadratae, ad 12-14 μ x 8 μ.

Caetera ignota.

Plants small to medium size, dendroid, light green, glossy, growing in erect masses on trees; primary stems stoloniferous, creeping, slender, naked; secondary stems 1-2 cm. long, erect, loosely pinnately branched, the branches to 1.3 cm. long, stipe woody, 5-10 mm. long; branch leaves crowded, imbricated to erect spreading, in five series, deeply concave plane, 1-2 mm. long, 0.7-1 mm. wide, broadly ovate to ovate-lanceolate, apiculate or short acuminate, the margins entire or serrulate above; costa single, slender, ending at mid-leaf or somewhat above; secondary stem leaves like those of the branches, sometimes larger, to 2.6 mm. long; stipe leaves squarrose-recurved, very small, 0.8-1.4 mm. long, 0.8-1 mm. broad, distant, plane, triangular-ovate, abruptly short acuminate, the margins entire or serrulate; costa single, weak, sometimes branching at the apex, ending above the middle, or short and double, rarely absent; cells of all leaves linear in outline, more or less thick-walled, often with a single small papilla at each end, median cells 25-50 μ x 3-6 μ, the apical cells short, oval to rhomboidal 15-32 μ x 3-12 μ, cells at the insertion 18-40 μ x 3-11 μ, alar group poorly defined, the cells few, yellow, incrassate, irregular to subquadrate, 12-14 μ x 8 μ.

Sporophyte unknown.

Type locality: Haiti, en route Savanne Zombi, *Mackaness,* 1944.

General distribution: Haiti, and the Dominican Republic.

Illustrations: Plate 20.

West Indies. HAITI: Hardwood patch en route Savanne Zombi, Mornes des Commissaires, Apr. 28, 1944, *Faith P. Mackaness* 99, *Type* (BART). DOMINICAN REPUBLIC: La Vega, Loma Campana (deep ravine of Rio Los Platanos), 1036 m. alt., Jan. 2, 1948, *H. A. Allard* 18382a (MICH).

The distinguishing characteristics of this species are its small squarrose stipe leaves, and the broadly ovate branch leaves which end in an apiculus. The closely related *P. cavifolia,* is easily separated by its appressed, ovate-lanceolate stipe leaves. See chart 1. *P. squarrosa* is at present known from only the original collection and another small collection from the Dominican Republic.

PIREELLA FALCIFOLIA Bart., Bryol. 49:117. 1946

Plants medium size to robust, dendroid, brownish or dark green, dull, growing in erect masses on trees; primary stems stoloniferous, creeping, slender, naked, leaves if present minute 0.4-0.5 mm. long, distant, closely appressed, lanceolate, ecostate; secondary stems 3-6 cm. long, erect, reddish, dendroid, thickly pinnately branched, the branches to 1.8 cm. long, the woody stipe 7-16 mm. long; branch leaves crowded, mostly falcate secund, weakly ranked, concave, 2.5-4 mm. long, 1.2 mm. wide below, lanceolate to ligulate-lanceolate, acuminate, margins entire or serrulate above; costa single, slender, percurrent; secondary stem leaves like the branch leaves, usually larger, 4-5 mm. long, 1.5 mm. wide below, abruptly lanceolate from an ovate base, acuminate, percurrent; stipe leaves scale-like, appressed, 1.8-2 mm. long, 1.2 mm. wide, lanceolate, entire, costa weak, ending at mid-leaf or a little beyond; cells of all leaves linear in outline, rather short, incrassate, smooth, rarely porose, median cells 16-30 μ x 3-6 μ, the cells of the apical region oval-rhomboidal in outline, 10-28 μ x 3-8 μ, basal cells short, 15-25 μ x 4-12 μ, yellow, porose, the alar region poorly defined; the cells few, irregular to subquadrate, 6-15 μ x 6-12 μ, yellow.

Gemmae common, septate, 3-12 cells long, uniseriate, in the axils of leaves.

Sporophyte unknown.

Type locality: Guatemala, Dept. Zacapa.

General distribution: Guatemala.

Illustrations: Bart., Mosses of Guatemala, *f.* 116, A-D; Plates 14, 15.

Central America. GUATEMALA: Zacapa, cloud forest in ravine bordering Quebrada Alejandria, summit of Sierra de Las Minas, vicinity of Finca Alejandria, 2500 m. alt., Oct. 13, 1939, *J. A. Steyermark* 29868, *Type* (F, BART, MICH, UC).

This striking species is known from only the type collection, and is clearly different from any other member of the genus by its appressed stipe leaves, and the lanceolate to ligulate branch leaves which are mostly falcate secund.

PIREELLA POHLII (Schwaegr.) Card., Rev. Bryol. 40:18. 1913

Leucodon pohlii Schwaegr., Suppl. III. 2. tab. 232. *Neckera longipes* C.M., Syn. 2:123. 1851. *Neckera pohlii* C.M., Syn. 2:114. 1851. *Pterobryum pohlii* Mitt., Linn. Soc. London, Journ. Bot. 12:426. 1869. *Pilotrichum ludoviciae* Jaeg., in Jaeg. & Sauerb. Ber. St. Gall. Nat. Ges. 1874-75:158. 1876. *Pilotrichella longipes* (C.M.) Jaeg., *op cit.* 158. *Pilotrichum longipes* Mont in Hb. *Neckera ludoviciae* C.M., Flora 58:534. 1875. *Pilotrichum tonduzii* Ren. & Card., Soc. Roy. de Bot. de Belg. Bul. 31(1):184. 1893. *Pilotrichella tonduzii* Ren. & Card. in Hb. *Pterobryum ludoviciae* (C.M.) Broth., in E. & P. Nat., Pflanzenf. ed. 1. 1(3):796. 1906. *Pirea ludoviciae* (C.M.) Broth., *op. et loc. cit. Pirea tonduzii* (Ren. & Card.) Broth., *op. et loc. cit. Pirea pohlii* (Schwaegr.) Herzog, Bot. Centralbl. Beihefte, Abt. B. 26(2):88. 1909. *Pirea papillosula* Card., Rev. Bryol. 37:6. 1910. *Pireela papillosula* (Card.) Card., Rev. Bryol. 40:18. 1913. *Pireella tonduzii* (Ren. & Card.) Card., *op. et loc. cit. Pireella ludoviciae* (Ren. & Card.) Card., *op. et loc. cit.*

Schwaegrichen's original material was not available for study. His original description (1828), follows:

"Caulis uncias duas, ad quatuor longus, simplex, rarius divisus, tenuissimus, filiformis, fuscus; rami sesquiunciales aut biunciales, basi indivisi, apice ramosi, ramulis pinnatis vel subsimplicibus, fasciculatis. Folia sparsa, parum patula, oblonga, apice sesim in mucronem praelongum attenuata, valde concava, nisi maxime augentibus lentibus lustrata integerrima, obscurius viridia: nervo ultra mediam partem evanescente, debili, concolori: cellulis sublinearibus. Flores dioeci: masculi in minoribus caulibus et ramia nati, folio caulino duplo breviores, oblongi, acuminati: folia ovato-acuminata, obliqua, enervia: antheras copiosae, teretiusculae, virides, demum albescentes: paraphyses filiformes, limpidae. Vaginula capsula paullo longior, teres, fusca. Pedunculus uncialis, erectus, interdum subflexuosus, badius. Capsula aequalis, ovata, laevis, badia. Peristomium orificii diametro dimidio paullo minus, constat e dentibus geminatis, inter se connatis, ut opus quoddam reticulatum sistant; finduntur irregulari modo hic illuc, sunt caeterum crassi, transversim striati, lurido-lutescentes. Operculum e conico rostratum, erectum aut parum curvulum, capsulae longitudine ferrugineum. Calyptra deest."

The following description was drawn from authentic material:

Plants medium size, dendroid, dark green to pale green, usually dull, growing in erect masses on trees and logs; primary stems stoloniferous, very slender, naked, leaves when present distant, minute, 0.4-0.5 mm. long, ecostate, scale-like, lanceolate, appressed; secondary stems 2-8 cm. long, erect, dendroid, with a woody stipe, 4-7 mm. long below, pinnately branched, the branches short, 1.5 cm. long, often becoming flagelliform, to 6 cm. long, bearing closely imbricated, minute, lanceolate, scale-like, ecostate, leaves to 0.5 mm. long, the flagelliform branches often rooting and developing ordinary leaves at their apices; branch leaves crowded, imbricated to erect spreading, in five distinct, spiral rows, deeply concave, 1.5-2.5 mm. long, 0.8-1.4 mm. wide, oblong-lanceolate, auriculate or the basal angles rounded, short acuminate, margins serrulate above or to the base, or entire; costa single, slender, percurrent or nearly so; cells long linear in outline, sinuous, thin-walled, seldom porose, often with a single papilla on each end wall, the papillae most frequent in the upper half on back of the leaves, rarely throughout; median cells 25-48 μ x 3-6 μ, the cells of the apical region similar to somewhat shorter, 18-40 μ x 3-8 μ, the basal cells shorter and broader, 20-32 μ x 4-14 μ, porose, yellow, the alar region poorly defined, of a few irregular to subquadrate, thin-walled, cells forming a rounded cluster in each auricle, the cells 3-7 μ in diameter or 8-20 μ x 8-18 μ; leaves of secondary stems, and the stipe similar to those of the branches, usually larger, to 3.5-4 mm. long, 2 mm. wide, the auricles often well developed.

Dioicous. Male and female inflorescences on short lateral branches; perichaetia 1-2.5 mm. long, the leaves 0.8-2.5 mm. long, lanceolate, gradually narrowed to a long linear, serrulate, point; costa single, slender, ending in the apex, areolation as in the ordinary leaves; perigonia very small, to 0.8 mm. long, the leaves ovate, ecostate, entire; capsule exserted, erect, oval-cylindric, brown, 2-2.25 mm. long, 0.5-0.8 mm. wide, narrowed at the mouth; seta 8-10 mm. long, smooth, erect, reddish; operculum conic-rostrate, the beak about 1 mm. long, pointed; peristome double, the teeth of the exostome lanceolate, smooth, often perforate at the base, very irregular, 0.20 mm. long, the endostome rudimentary, consisting of a few fragments attached to the exostome; spores rounded, smooth or finely papillose, 22-30 μ in diameter; calyptra cucullate, 2 mm. long, pilose.

Gemmae frequent, 3-12 cells long, uniseriate, in axillary clusters on the stem.

Type locality: Brazil. *D. Pohl* 4.

General distribution: Louisiana, Florida, Mexico, Central America, and the West Indies.

Illustrations: Schwaegr. Suppl. III, *Tab.* 232. *f.* 1-15; Grout, Moss Fl. N. Amer. *pl.* 57, 1-5; Plates 28, 29.

United States. LOUISIANA: The type of *Neckera* (*Orthostichella*) *ludoviciae* C.M. *C. Mohr.* (NY). FLORIDA: Sanford (on trunks of elm, as *Pilotrichum ludoviciae*), Apr. 12, 1903, *S. Rapp* (NY).

Mexico. VERACRUZ, El Cerro de San Gabriel, vicinity de Pueblo Nuevo, 4 km. north of Campo Experimental de Hule, El Palmar, Zongolica, Dec. 15-16, 1944, *J. V. Santos* 3899 (MICH).

Central America. GUATEMALA: Peten, Fallabón—Yaxha Road (as *Pterobryum angustifolium*), Mar. 22, 1933, *C. L. Lundell* 2041 (FH, MICH, BART). EL SALVADOR: Finca San Nicolás, 1923, *S. Calderón* 1768 (US). COSTA RICA: Boruca (no data), 1891, *Tonduz* 622? (the original material of *Pilotrichum* (*Pilotrichella*) *tonduzii*) (PC).

West Indies. JAMAICA: Westmoreland Hills, New Market, Woodstock, near Beaufort (as *Pireella ludoviciae*), Sept. 21, 1906, *E. G. Britton* 571 (NY, US). HAITI: L'Artibonite, section Dessaliues, west of Caille la Croix, vicinity of St. Michel de l'Atalaye, 350 m. alt., Dec. 11, 1925, *E. C. Leonard* 7991 (F, US, FH). MARTINIQUE: Source Sulfurense des Deux-Choux (epiphyte à la base des arbres en forêt dense et humide), 680 m. alt., Jan. 5, 1941, *M. & M. Stehlé* 3861 (NY, BART). TRINIDAD: Moruga (the type of *Neckera* (*Orthostichella*) *longipes*, Crüger 1347 (NY).

BRAZIL: *D. Pohl*, 4:Type, not seen.

This is a very common species, distinct from all others because of its dendroid habit, its appressed stipe leaves, its secondary stem and branch leaves auriculate or rounded at the basal angles, the elongate outline of the cells of all leaves, and the poorly defined alar regions. *P. cymbifolia* is closely related to this species, but the former has a tufted habit, numerous rows of quadrate alar cells, and clasping leaves which are never auriculate (see chart 1). The sporophytes of the two species are almost identical.

P. pohlii exhibits a small degree of variability in the stature of the plants, the leaf shape, and degree of papillosity of the cells. These minor variations were the basis for Renauld and Cardot's species *P. tonduzii* (1893), and *P. papillosula* (1910). Renauld and Cardot (1893) described *P. tonduzii* merely as having "foliis sub-acuminatis, acuti, cellulis ellipticis oblongisve, nervisque dorso haud vel vix cristatis parce denticulatis vel sublaevibus." Later Cardot (1910) recognized the similarity between *P. papillosula* and *P. pohlii* and described the former species as "A *P. cymbifolia* (Sull.) Broth. et *P. pohlii* (Schwaegr.) Broth. statura pygmaea (caule secundario 1-2 centim. alto) foliis brevioribus, triangulari-lanceolatis, marginibus e basi leviter sed sat distincte serrulatis, cellulisque in pagina dorsali papilla minuta prominula apice notatis facile distinguitur. Fructus ignotus." The variations in the appearance and the development of papillae, size of plants, serration of leaves, as well as their width and the degree of acumination, are all characters eminently within the admitted limits of *P. pohlii*,

and slight differences based on any or all of these characters cannot be held as constituting sufficient basis for specific distinction. Müller (1875), in describing *Neckera* (*Orthostichella*) *ludoviciae* and *Neckera longipes*, did not draw any distinctions between his plants and *P. pohlii*. The plants of the original collections of his two species, compared with the original description of *Leucodon pohlii*, and later authentic material, show absolutely no major differences. Therefore, there are no bases for keeping *P. ludoviciae* and *P. longipes* as valid species.

PIREELLA CYMBIFOLIA (Sull.) Card., Rev. Bryol. 40:17. 1913

Pilotrichum cymbifolia Sull., in A. Gray's Man. Ed. 2, 681. 1856. *Pterobryum cymbifolia* (Sull.) Sull., Icon. Musc. 1:122. Tab. 76. 1864. *Pilotrichella cymbifolia* (Sull.) Jaeg. & Sauerb. Ber. St. Gall. Nat. Ges. 1874-75:158. 1876. *Neckera cymbifolia* (Sull.) C.M., Lesq & James Man. 284. 1884. *Pirea cymbifolia* (Sull.) Broth., in E. & P. Nat. Pflanzenf. ed. 1. 1(3):796. 1906. *Pireella denticulata* Card. & Thér., Rev. Bryol. 40:17. 1913.

Plants small to medium size, light green, glossy, growing on trees or logs in lax tufts, rarely dendroid; primary stems creeping, filiform, usually naked, leaves when present, distant, minute, 0.5 mm. long, lanceolate, appressed; secondary stems 2-7 cm. long, irregularly and sparingly branched, tufted, or occasionally dendroid from a woody stipe 4-7 mm. long; flagelliform branches frequent, to 7 cm. long, bearing minute, appressed leaves, to 0.4 mm. long; branch leaves crowded, imbricated to erect spreading, distinctly five-seriate, deeply concave, 1.5-2 mm. long, 0.8-1 mm. wide, clasping, never auriculate, oblong-lanceolate, short acuminate, margins serrulate above or nearly to base, or entire; costa single, slender, percurrent; cells long, narrowly linear in outline, thin-walled, seldom porose, with a small papilla on each end wall, the papilla localized on the backs of the cells in the upper half of the leaf, or rarely throughout the leaf; median cells 27-50 μ x 4-6 μ, the cells of the apical region often the same size, but mostly 32-40 μ x 4-6 μ, basal cells shorter, 20-32 μ x 4-12 μ, yellow, porose, the alar region well defined, of 6-16 rows of yellow, sub-quadrate cells 6-16 μ x 4-10 μ, extending up the margin of the leaf, secondary stem leaves similar to the branch leaves, usually larger, to 3 mm. long.

Dioicous. Male and female inflorescences on short lateral branches; perichaetia small, to 1 mm. long, the leaves small, 0.8-1 mm. long, lanceolate, narrowly long-acuminate, ecostate, entire or serrulate above; perigonia very small, 0.8 mm. long, the leaves ovate to lanceolate, ecostate, entire, areolation as in ordinary leaves; capsules exserted, erect, oval-cylindric, brown, 2-2.25 mm. long, 0.5-0.8 mm. wide, slightly narrowed at the mouth; the seta 8-10 mm. long, smooth, erect, reddish, and flexuose; operculum conic-rostrate, the beak 1 mm. long, gradually acuminate; peristome double, the teeth of the exostome lanceolate, smooth, perforate, very irregular, 0.18 mm. long, the endostome rudimentary, consisting of transparent fragments attached to the exostome; spores round, brownish, finely papillose, 22-28 μ in diameter; calyptra cucullate, 2 mm. long, pilose.

Gemmae frequent, septate, 3-12 cells long, uniseriate, in axillary clusters on the stem.

Type locality: East Florida (from a tree on a hummock). *Binney.*

General distribution: Florida, Louisiana, Mexico, Central America, and the West Indies.

Illustrations: Sull., Icones Musc. *pl.* 76, *f.* 1-11.

United States. FLORIDA: East Florida (from a tree in a hummock, as *Pilotrichum cymbifolium*), 1846, *Binney, Type* (NY).

Mexico. YUCATAN: Chichen Itza (on floating stick in Scan Yui cenote), June 18, 1932, *W. C. Steere* 1403 (NY, MICH).

Central America. GUATEMALA: Alta Verapaz, Montana Yxocubvain, 2½ miles west of Cubilgüitz (on dead log at summit of mountain), 300-500 m. alt., Mar. 12, 1942, *J. A. Steyermark* 44995b (F, BART, MICH). BRITISH HONDURAS: Yucatan Peninsula, Subun River, Gracie Rock, May 29, 1935, *P. H. Gentle* 1659 (F, FH, MICH). HONDURAS: (no data, from C. F. Millspaugh), Sept. 1899, *Gaumer* 1213a (NY).

West Indies. BAHAMA ISLANDS: New Providence (the type of *Pilotrichella cymbifolia*), *E. G. Britton* 6569 (NY). JAMAICA: Montego Bay, July 1922, *E. Jaderholm* 128 (NY). CUBA: Pinar del Río, Sierra la Guira, San Diejo de los Banos, Apr. 21, 1914,

Bro. Leon 4500 (NY). HAITI: Tortue Isand, vicinity of La Vallee, Dec. 28, 1928-Jan. 9, 1929, *E. C. Leonard & G. M. Leonard* 11613b (NY, FH, MICH). PUERTO RICO: Rio Abajo Planting Project, south of Arecibo (on *Diospyros*), Feb. 23, 1940, *W. C. Steere* (FH, MICH).

P. cymbifolia is readily identified by the tufted scraggly habit, leaf cells linear in outline, and numerous rows of quadrate alar cells extending up the leaf margin. It has often been confused with *P. pohlii*, which also has linear cells throughout the leaf, but the latter species is typically dendroid, with a conspicuous woody stipe which bears appressed leaves. The alar region of the leaf in *P. pohlii* is poorly defined, and is composed of only a few sub-quadrate cells in a cluster in the basal angles of the leaf. These are rounded to auriculate in contrast to those in leaves of *P. cymbifolia* which are never auriculate, but merely clasping. See chart 1.

P. cymbifolia varies in its manner of branching and degree of papillosity of cells of the leaf, but not to any great extent in the other characters of the stem. Some of the plants in any tuft may be laxly frondose, and exhibit a suggestion of a stipe, but in none of these variants is there any major variation in the other characters. The plants of the collections from British Honduras and Yucatan have strongly papillose leaves; however, the presence and degree of development of papillae appears to be a variable character.

Cardot and Thériot (1913), proposed the species *P. denticulata* as distinct from *P. cymbifolia* chiefly on the basis of the degree of denticulation of the leaves, and the original description says merely, "*P. cymbifolia* (Sull.) simillima, differt foliis latiore et breviore acuminatis, fere e basi denticulatis, cellulis alaribus magis numerosis." In the original material only a very few leaves are denticulate to the base, a situation not uncommon in *P. cymbifolia*. The degree of denticulation varies somewhat in each leaf examined; therefore, this does not appear to be a very reliable character on which to base a species.

EXCLUDED

Pterobryopsis subcochlearifolia Thér. in Hb., is *Pilotrichella flexilis* (Hedw.) Jaeg.

Pterobryopsis dentata Thér. Trav. Bryol., Musci Hispan. 18-19. 1944, belongs to the genus *Meteorium*.

Renuldia subpilifera Williams, Journ. Washington Acad. Sci. 20(10):176. 1930, is *Pilotrichella cuspidans* Ren. & Card.

REFERENCES

BESCHERELLE, É. 1876—Florule bryologique des Antilles françaises. Ann. Sci. Nat. VI, Ser. 3:175-265.

BRIQUET, J. 1935—The International Rules of Botanical Nomenclature. 1-151. Ed. 3. Gustave Fischer.

BRITTON, ELIZABETH G. 1918—Jaegerinopsis squarrosa, n. sp. Bryologist 21:48-50.

BROTHERUS, V. F. 1906—Musci. *In* Engler & Prantl, Die Natürlichen Pflanzenfamilien. Ed. 1. 1(3):142-1246.

———1924-25—Musci. *In* Ibid. Ed. II. 10:1-479; 11:1-542. Leipzig.

CARDOT, J. 1893—Musci costaricenses. Soc. Roy. de Bot. de Belg. Bul. 32(1):175-177.

———1910-1913—Diagnoses préliminaires de mousses mexicaines. Rev. Bryol. 37:4-13, 49-59, 65-72, 117-128; 40:33-40.

CRUM, H. A. 1951—The Appalachian-Ozarkian element in the Mexican mosses. Unpublished Ph.D. dissertation. Microfilmed in University of Michigan Library.

FLEISCHER, M. 1900-1922—Die Musci der Flora von Buitenzorg. 4 vols. Leiden.

———1906—Neue Familien, Gattungen und Arten der Laubmoose. Hedwigia 45:53-87.

HEDWIG, J. 1801—Species muscorum frondosorum 1-352, Tab. 1-77.

HORNSCHUCH, F. 1840—Musci *In* Martius', Flora Brasiliensis 1(2):1-99. 4 tables.

JAEGER, A. & Ö. F. SAUERBECK 1877-1878—Genera et species muscorum systematice

disposita seu Adumbratio florae muscorum totius orbis terrarum. Ber. St. Gall. Nat. Ges. 1877-1878:257-514.

KINDBERG, N. C. 1899—Studien über die Systematic der pleurokarpischen Laubmoose. II. Bot. Centralbl. Beihefte, Abt. B. 77(2):49-55.

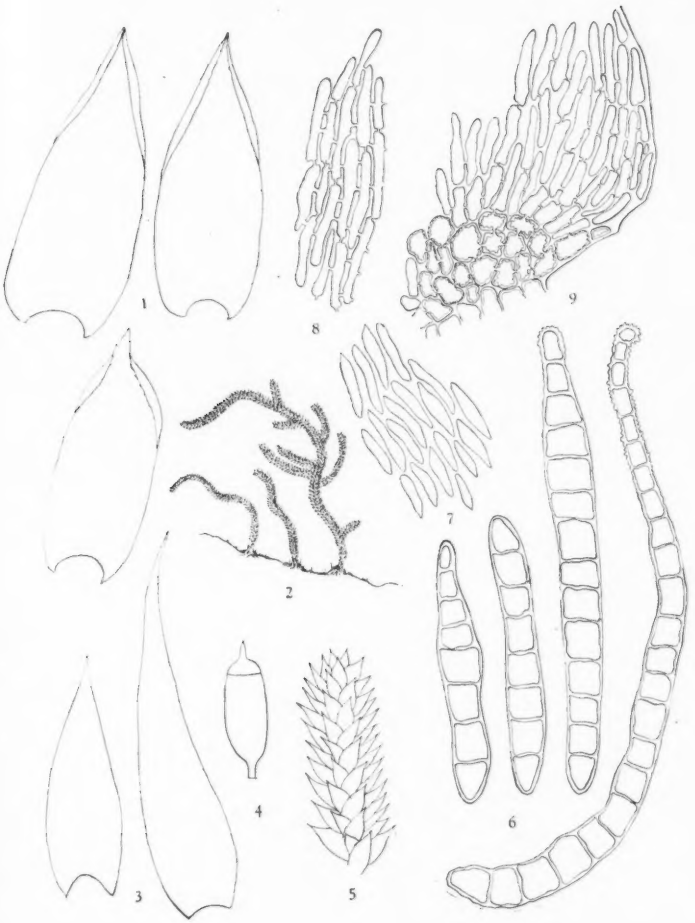
MITTEN, W. 1869—Musci Austro-Americani. Enumeratio muscorum omnium austro-americanorum auctori hucusque cognitorum. Linn. Soc. London, Journ. Bot. 12:1-659.

SCHLECHTENDAL, D. DE & AD. DE CHAMISSO 1831—Plantarum mexicanum a cel. viris Schiede et Deppe collectarum recensio brevis. Linnaea 6:22-64.

SCHWAEGRICHEN, F. 1828—Supplements I-IV to J. Hedwig. Species muscorum frondosorum. Leipzig.

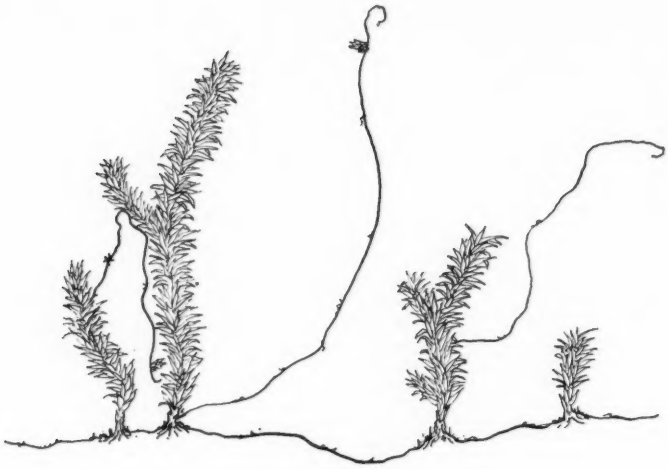
VERDOORN, F. 1950—The future of exotic cryptogamic botany. Bryologist 53:1-9.

PLATE 1

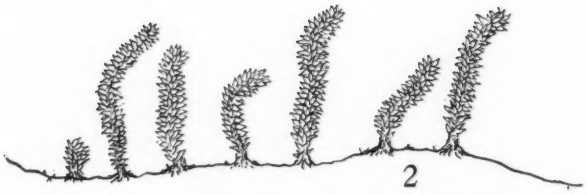


Figs. 1-9. *Orthostichidium pentagonum* (Hampe & Lor.) C.M.—1. Secondary stem leaves, ×120; 2. Habit sketch, ×5; 3. Perichaetial leaves, ×120; 4. Capsule, ×20; 5. Portion of branch, ×28; 6. Gemmae, ×675; 7. Cells of the apical region, ×675; 8. Median cells, ×675; 9. Alar region, ×675 (figs. 1-5, 7, 8 from the type).

PLATE 2



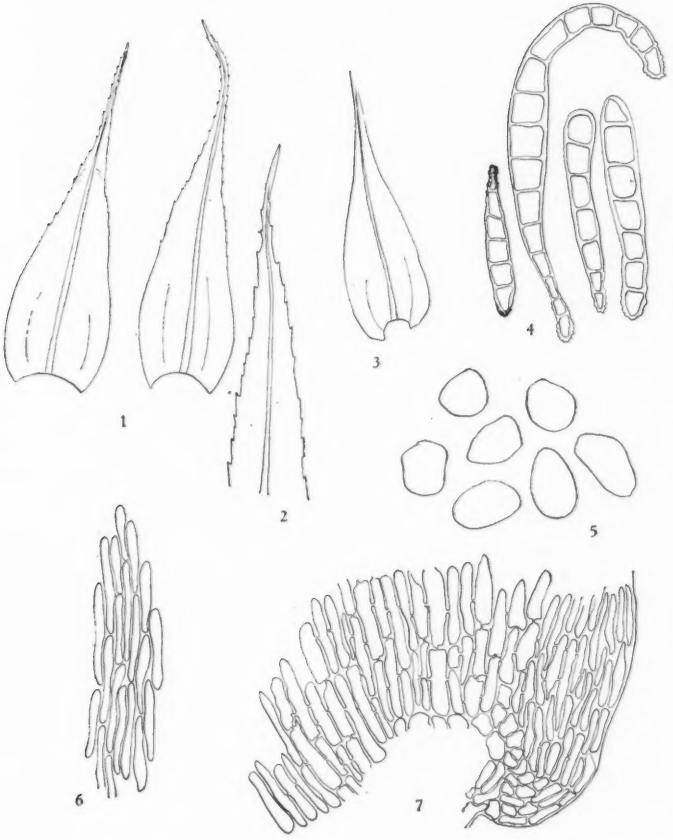
1



2

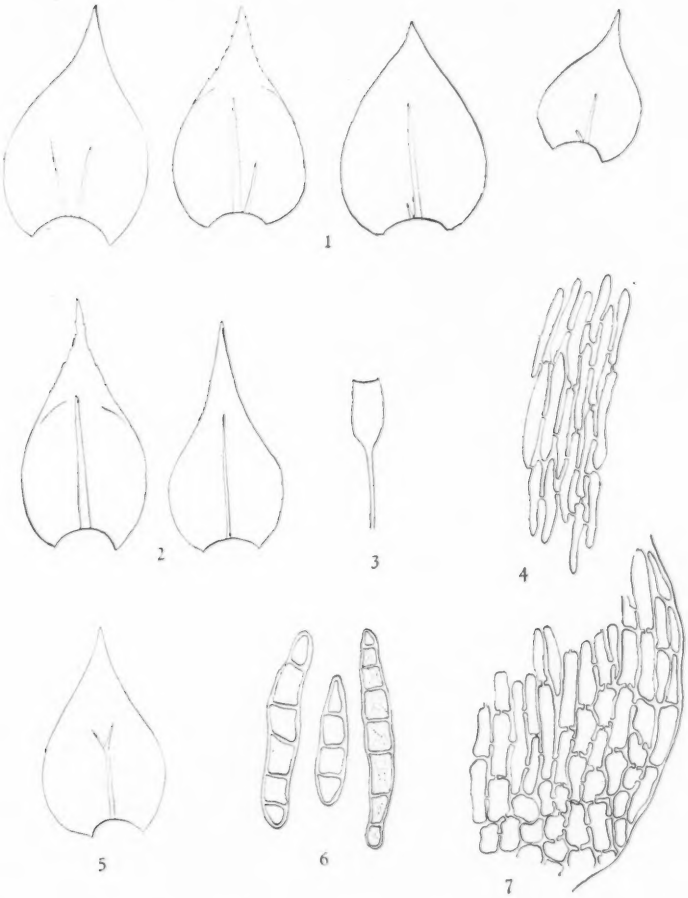
Figs. 1, 2. *Jaegerina*.—1. Habit sketch of *J. guatemalensis* Bart., ×3 (from type); 2. Habit sketch of *J. scariosa* (Lor.) Arzeni, ×3 (from type of *Jaegerinopsis squarrosa* E.G.B.).

PLATE 3



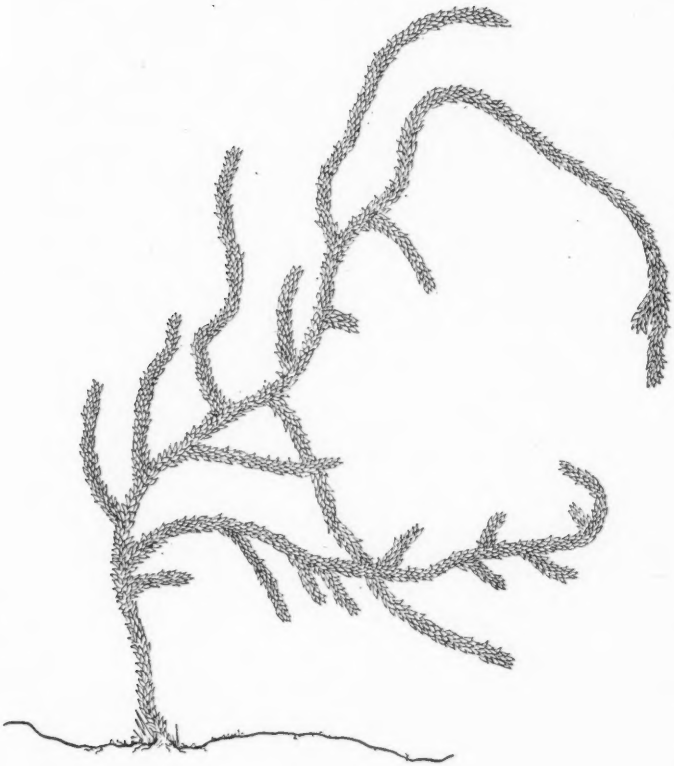
Figs. 1-7. *Jaegerina guatemalensis* Bart.—1. Leaves, ×28; 2. Leaf apex, ×165; 3. Leaf, ×28; 4. Gemmae, ×675; 5. Spores, ×675; 6. Median cells, ×675; 7. Alar region, ×675 (figs. 1-7 from the type).

PLATE 4



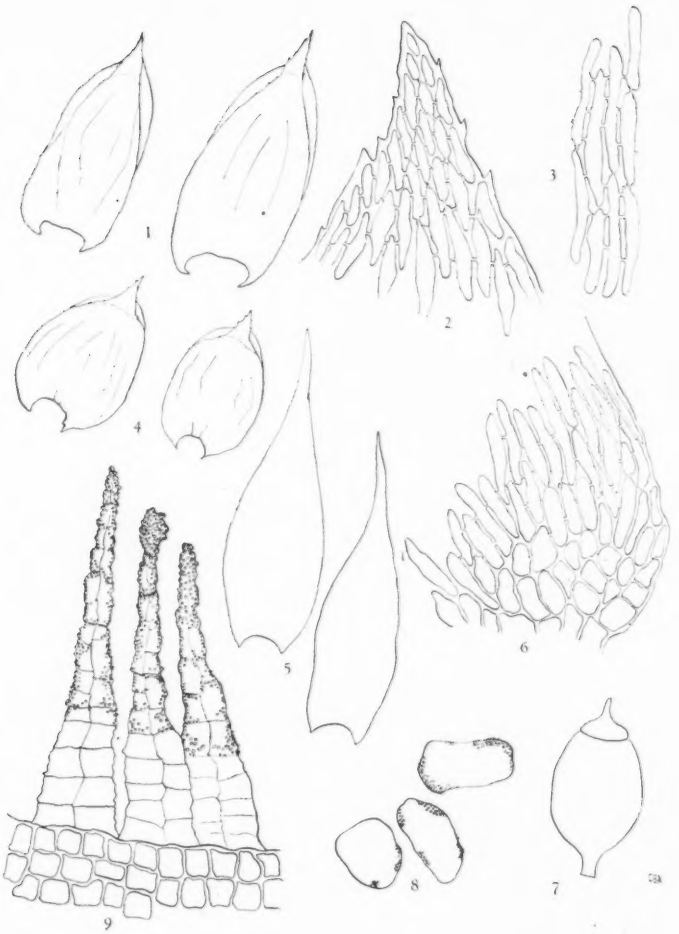
Figs. 1-7. *Jaegerina scariosa* (Lov.) Arzeni.—1. Leaves showing two costae, ×28; 2. Leaves with a single costa, ×28; Deoperculate capsule and seta, ×22; 4. Median cells, ×675; 5. Leaf with costa branching at apex, ×28; Gemmae, ×675; 7. Alar region, ×675 (figs. 1-7 from the type of *Jaegerinopsis squarrosa* E.G.B.).

PLATE 5



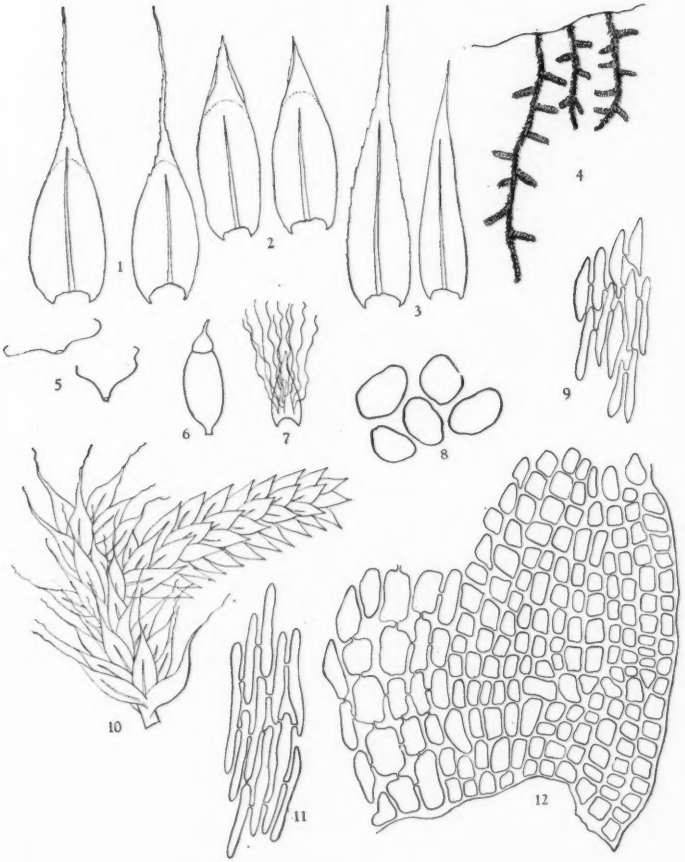
Renauldia mexicana (Mitt.) Crum.—Habit sketch, ×5

PLATE 6



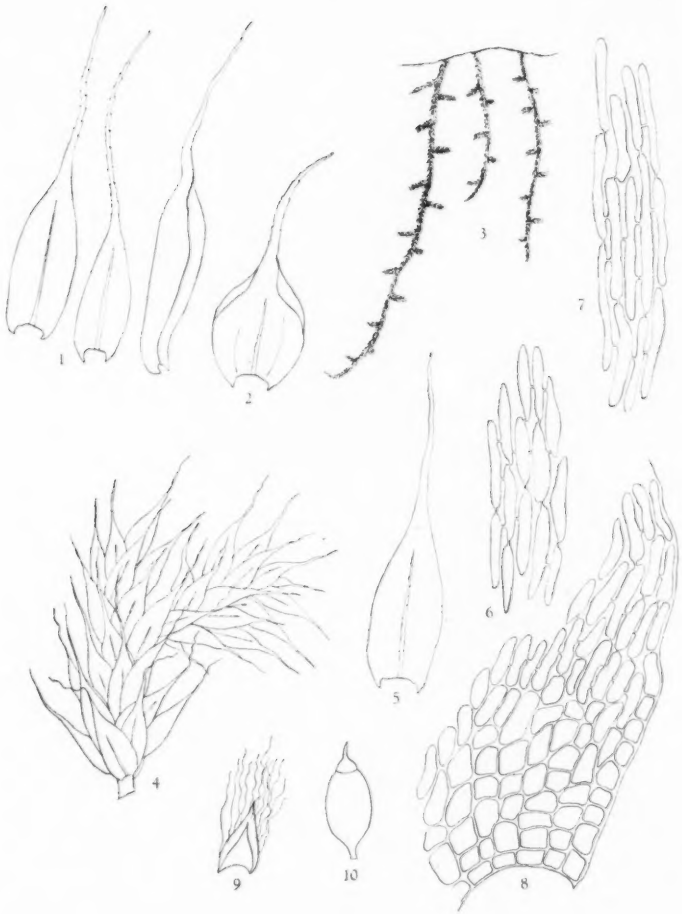
Figs. 1-9. *Renauldia mexicana* (Mitt.) Crum.—1. Secondary stem leaves, ×32; 2. Apex of leaf showing the apical cells, ×675; 3. Median cells, ×675; 4. Branch leaves, ×32; 5. Perichaetial leaves, ×32; 6. Alar region, ×675; 7. Capsule, ×30; 8. Spores, ×675; 9. Peristome, ×675 (figs. 1-6 from the type).

PLATE 7



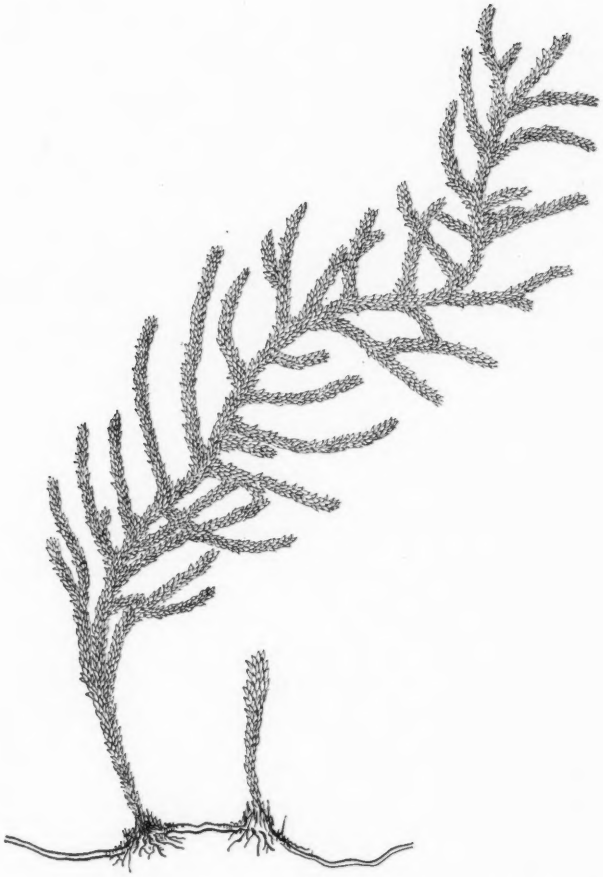
Figs. 1-12. *Orthostichopsis tetragona* (Hedw.) Broth.—1. Secondary stem leaves, ×30; 2. Branch leaves, ×30; 3. Perichaetial leaves, ×30; 4. Habit sketch, ×5; 5. Cross section of branch leaves, ×30; 6. Capsule, ×30; 7. Pilose calyptra, ×30; 8. Spores, ×675; 9. Cells of the apex of the leaf, ×1125; 10. Portion of secondary stem and branch, ×30; 11. Median cells, ×1125; 12. Alar region, ×1125 (figs. 1-12 from the type).

PLATE 8



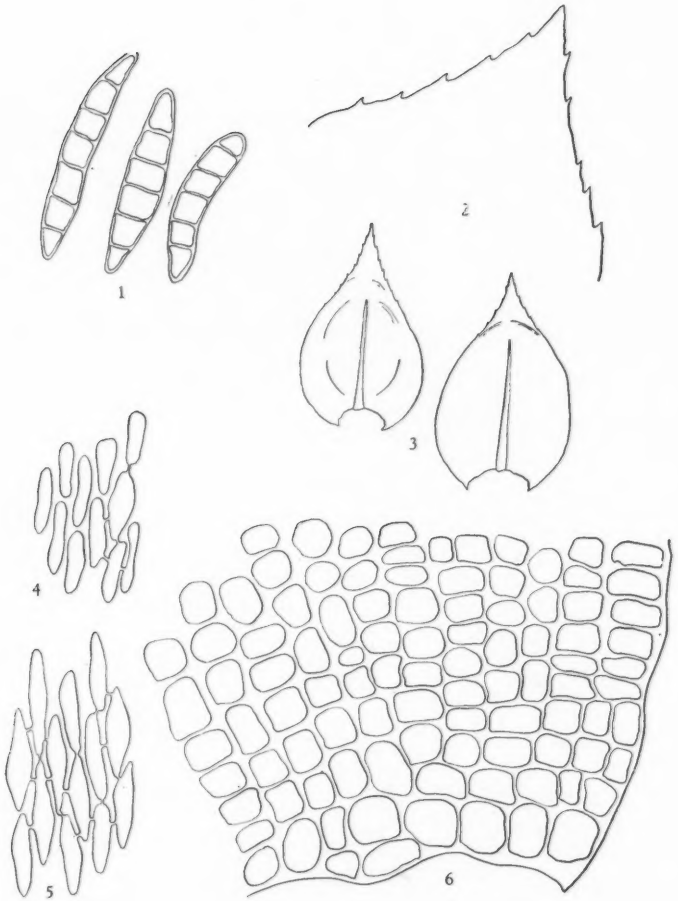
Figs. 1-10. *Orthostichopsis crinita* (Sull.) Broth.—1, 2. Secondary stem and branch leaves, ×30; 3. Habit sketch, ×5; 4. Portion of secondary stem and branch, ×30; 5. Perichaetial leaf, ×30; 6. Apical cells, ×1125; 7. Median cells, ×1125; 8. Alar region, ×1125; 9. Calyptra, ×30; 10. Capsule, ×30 (figs. 1-10 from the type).

PLATE 9



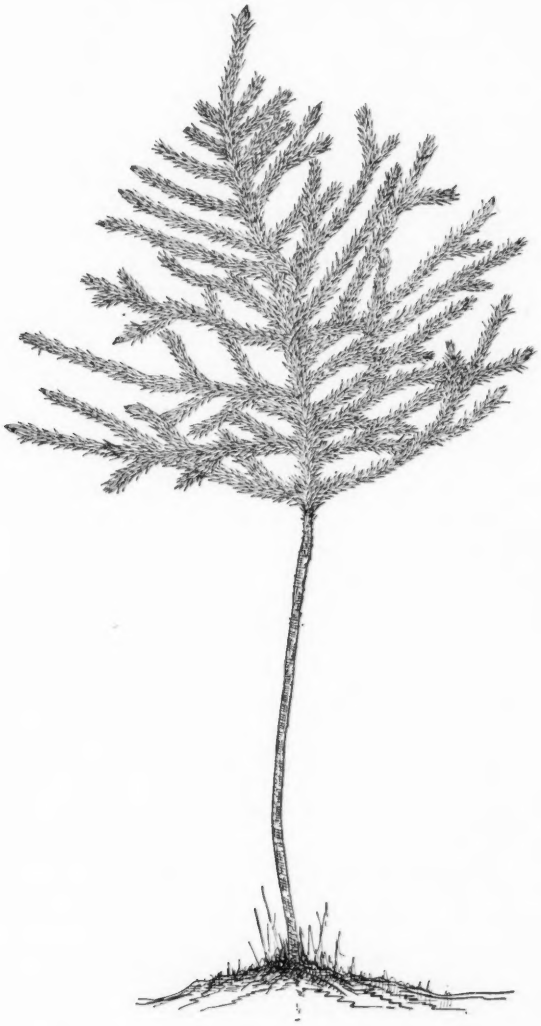
Pterobryopsis mexicana (Ren. & Card.) Fleischer.—Habit sketch, ×5

PLATE 10



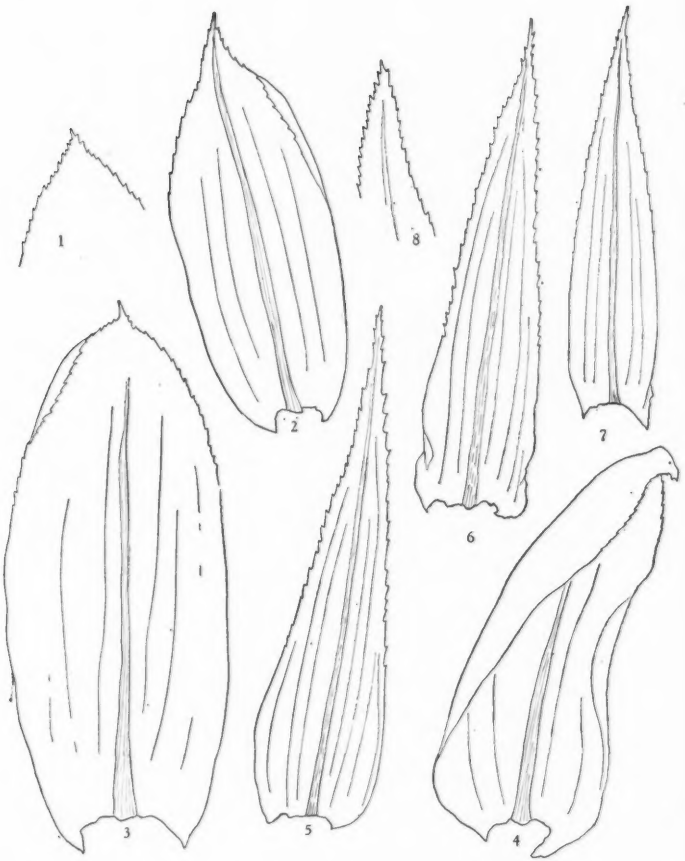
Figs. 1-6. *Pterobryopsis mexicana* (Ren. & Card.) Fleischer.—1. Gemmae, ×675; 2. Leaf apex, ×240; 3. Secondary stem and branch leaves, ×30; 4. Apical cells, ×1125; 5. Median cells, ×1125; 6. Alar region, ×1125 (figs. 2-6, from the type).

PLATE 11



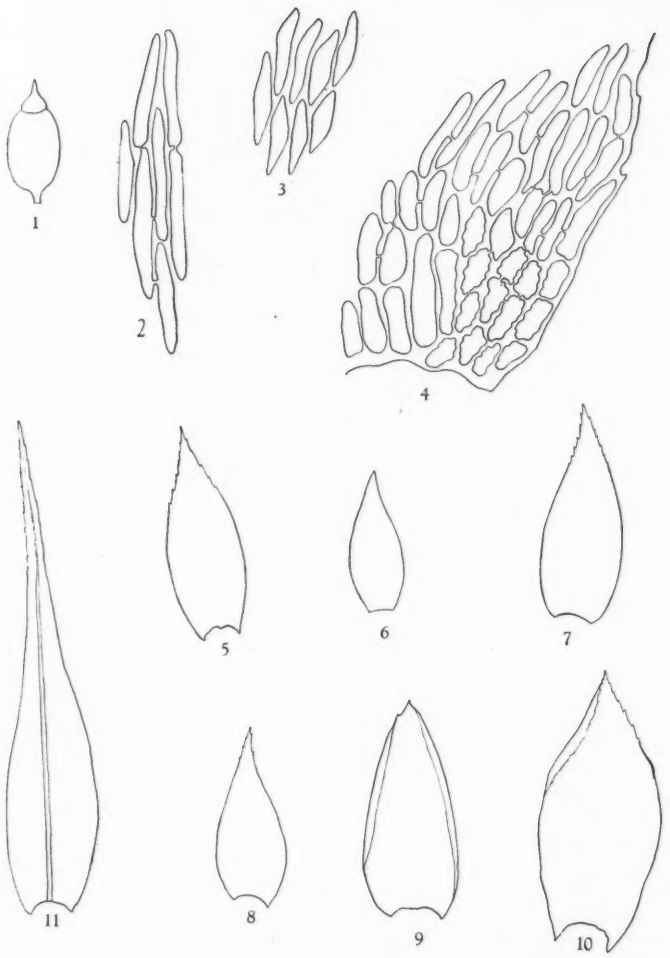
Pterobryon densum (Schwaegr.) Hornschuch.—Habit sketch, ×10

PLATE 12



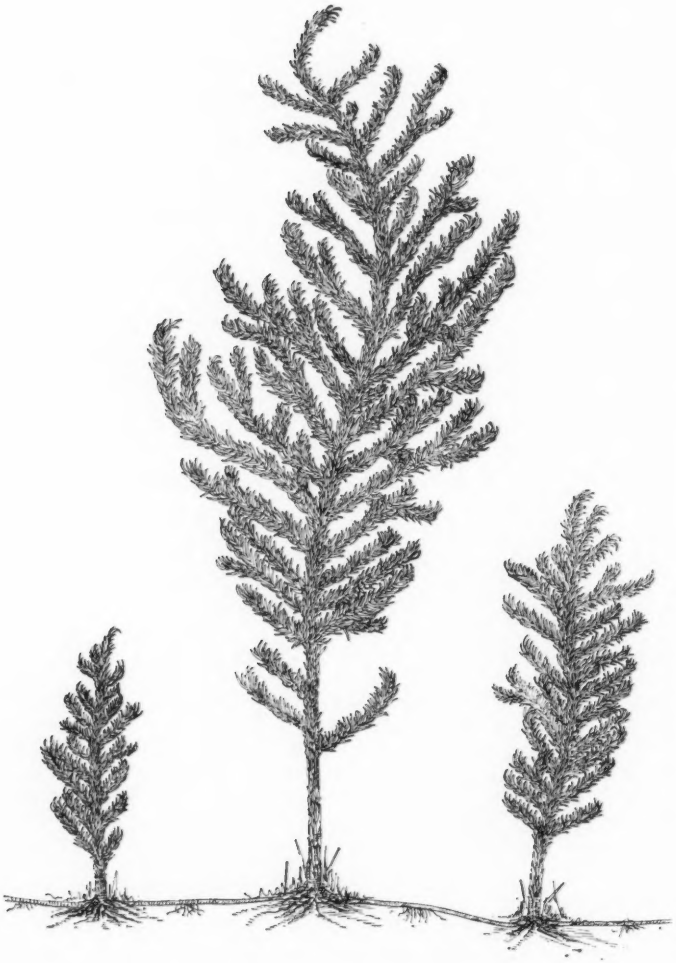
Figs. 1-8. *Pterobryon densum* (Schwaegr.) Hornschuch.—1. Leaf apex, ×12; 2-4. Broad ovate leaves rarely found in this species, ×12; 5-7 Typical leaves of this species, ×12; 8. Leaf apex of a typical leaf, ×12 (figs. 1-4, from the type of *P. panamense*).

PLATE 13



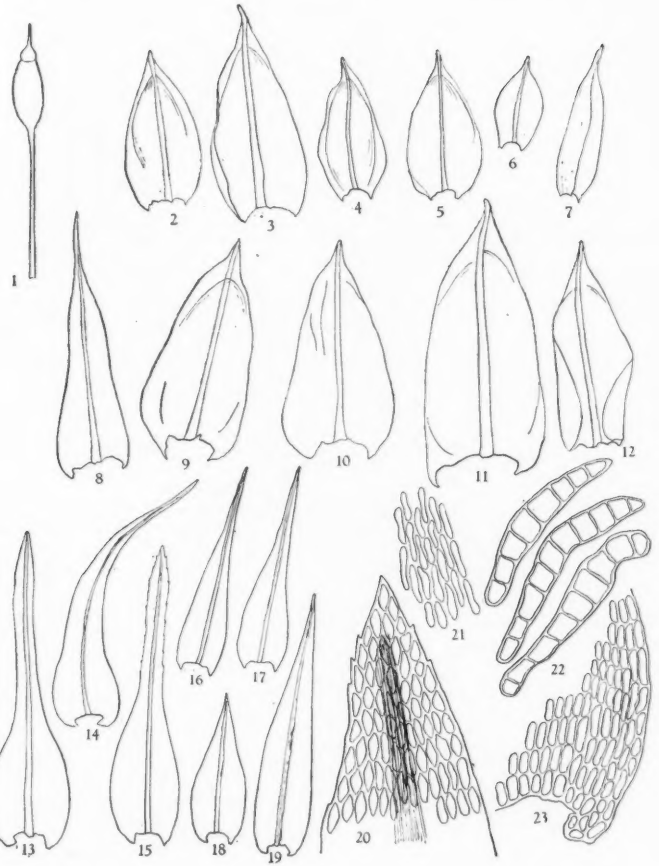
Figs. 1-11. *Pterobryon densum* (Schwaegr.) Hornschuch.—1. Capsule, ×28; 2. Median cells, ×1125; 3. Cells of the apical region of the leaf, ×1125; 4. Alar region, ×1125; 5-10. Perigonial leaves, ×30; 11. Perichaetial leaf, ×30.

PLATE 14



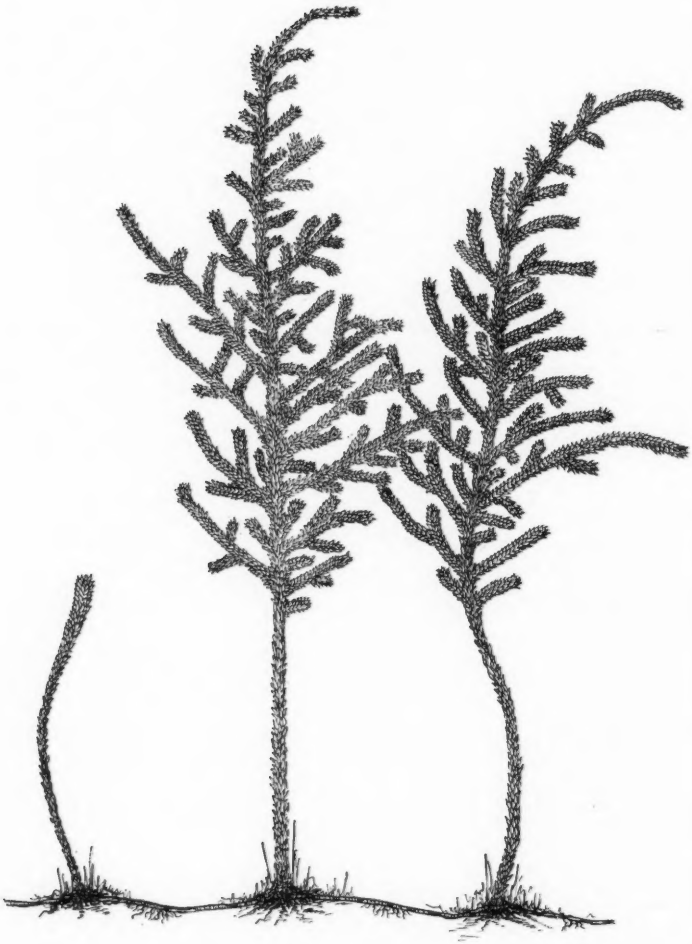
Pireella falcifolia Bart.—Habit sketch, ×8 (from the type)

PLATE 15



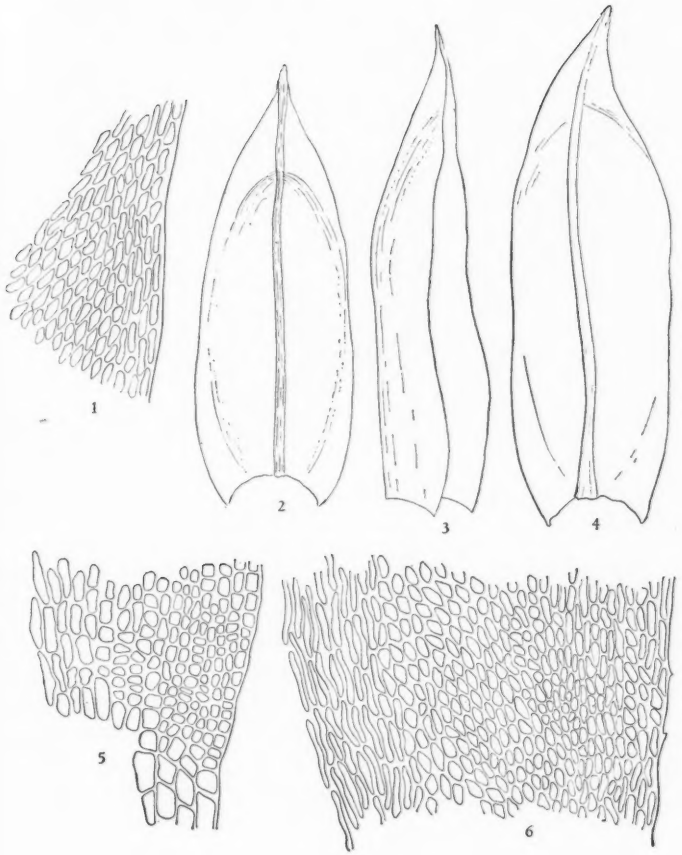
Figs. 1-23. *Pireella falcifolia* Bart.—1. sporophyte, ×25; 2-7. Branch leaves, ×125; 8. Perichaetial leaf, ×125; 9-12. Secondary stem leaves, ×125; 13, 14. Branch leaves, ×35; 15-19. Secondary stem leaves, ×35; 20. Leaf apex and cell of this region, ×675; 21. Median cells, ×675; 22. Gemmae, ×675; 23. Alar region, ×675 (figs. 1-23 from the type).

PLATE 16



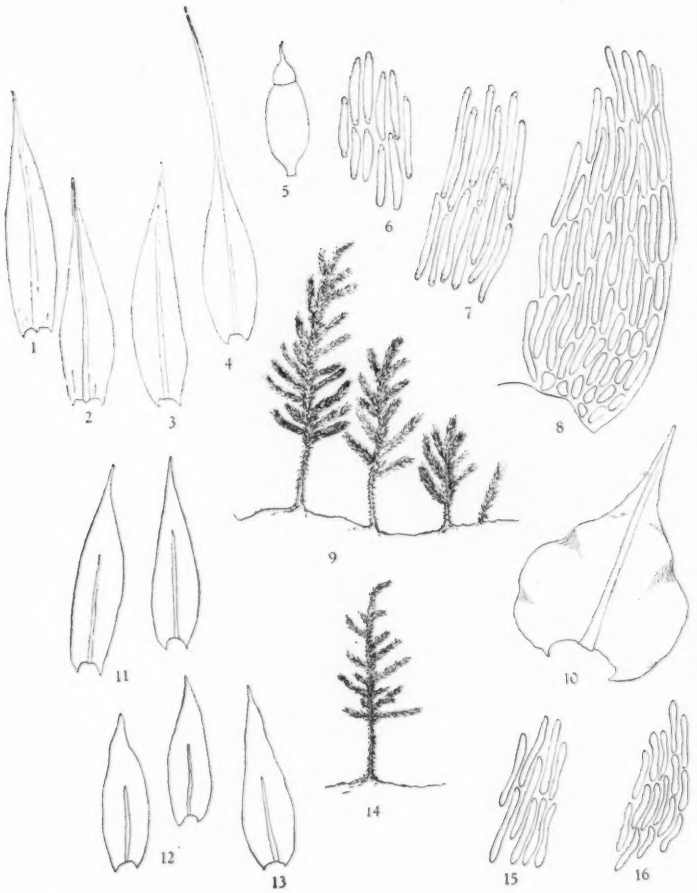
Pireella pachyclada (Ren. & Card.) Card.—Habit sketch, ×14 (from the type)

PLATE 17



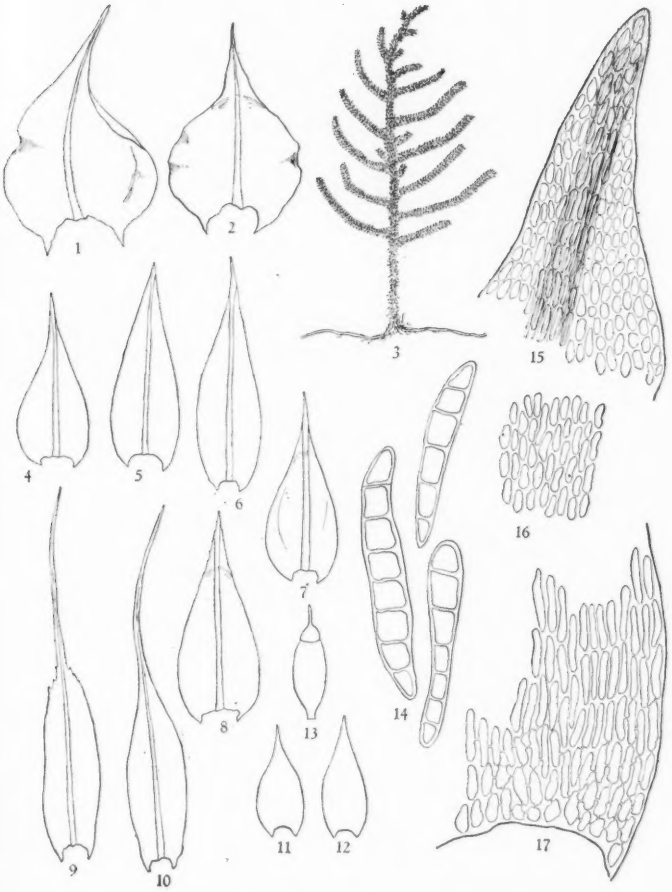
Figs. 1-6. *Pireella pachyclada* (Ren. & Card.) Card.—1. Cells of the apical region of the leaf, ×675; 2, 3. Branch leaves, ×125; 4. Stipe leaf, ×125; 5. Alar region, ×675; 6. Median areolation from the margin to the costa, ×675 (figs. 1-6, from the type).

PLATE 18



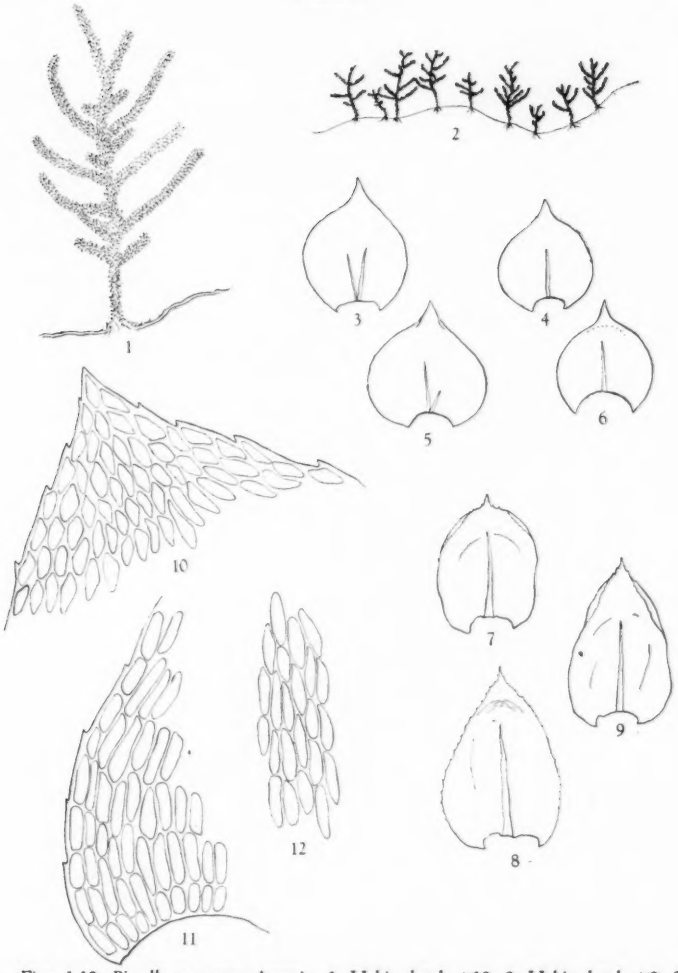
Figs. 1-16. 1-10. *Pireella angustifolia* (C.M.) Arzeni.—1, 2. Branch leaves, ×35; 3. Secondary stem leaf, ×35; 4. Perichaetial leaf, ×35; 5. Capsule, ×28; 6. Cells of the apex showing the single papilla at each end, ×675; 7. Median cells showing the single papilla at each end, ×675; 8. Alar region, ×675; 9. Habit sketch, ×5; 10. Stipe leaf, ×35; (all figs. except 9 from the type.) 11-16. *Pireella husnotiana* (Besch.) Card. 11-13. Branch leaves, ×35; 14. Habit sketch, ×5; 15. Median cells, ×675; 16. Cells of the apical region of the leaf, ×675 (figs. 11-16, from the type).

PLATE 19



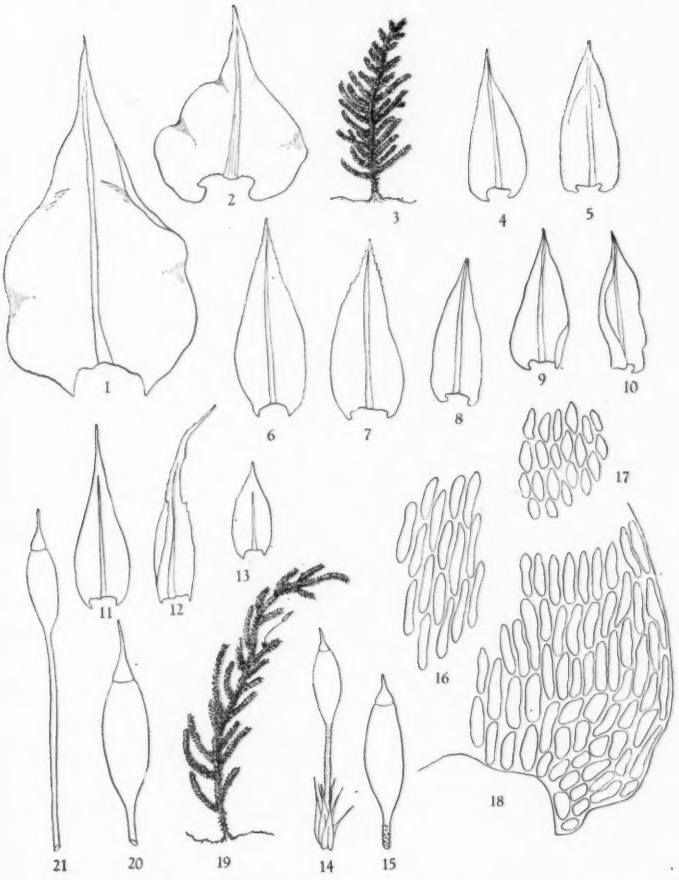
Figs. 1-17. *Pireella filicina* (Hedw.) Card.—1, 2. Stipe leaves, ×35; 3. Habit, ×9; 4-6. Branch leaves, ×35; 7, 8. Secondary stem leaves, ×35; 9, 10. Perichaetial leaves, ×35; 11, 12. Perigonial leaves, ×35; 13. Capsule, ×26; 14. Gemmae, ×675; 15. Leaf apex, and cells of the apical region, ×675; 16. Median cells, ×675; 17. Alar region, ×675 (figs. 1-13; 15-17, from the type).

PLATE 20



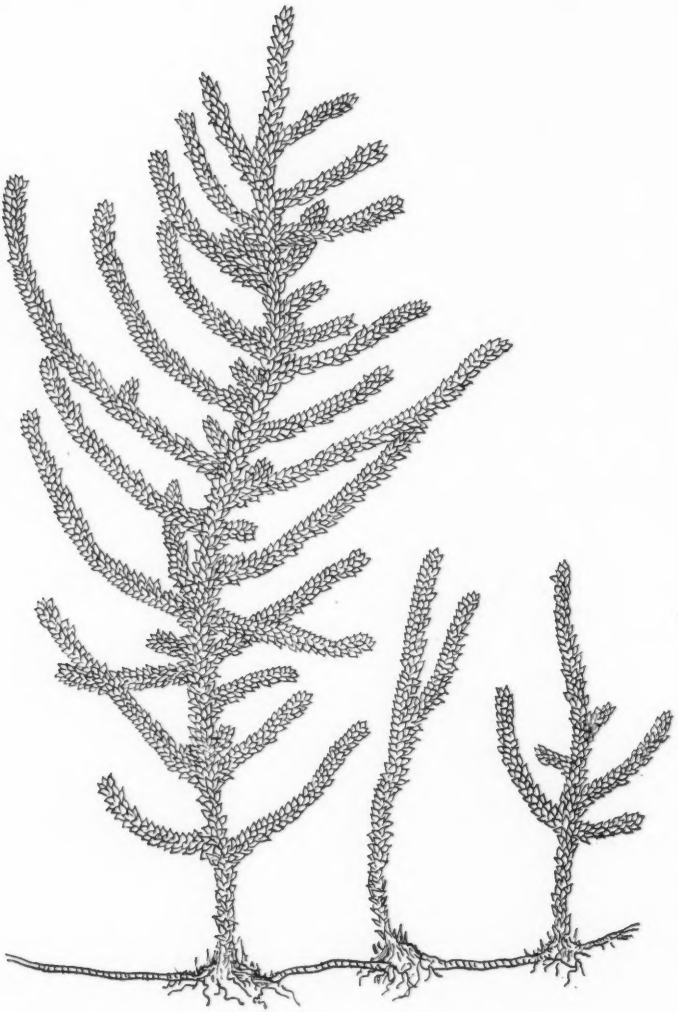
Figs. 1-12. *Pireella squarrosa* Arzeni.—1. Habit sketch, ×12; 2. Habit sketch, ×2; 3-6. Stipe leaves, showing variation in costa, ×35; 7, 8. Branch leaves, ×35; 9. Secondary stem leaf, ×35; 10. Leaf apex, and cells of the apical region, ×675; 11. Alar region, ×675; 12. Median cells, ×675 (figs. 1-12 from the type).

PLATE 21



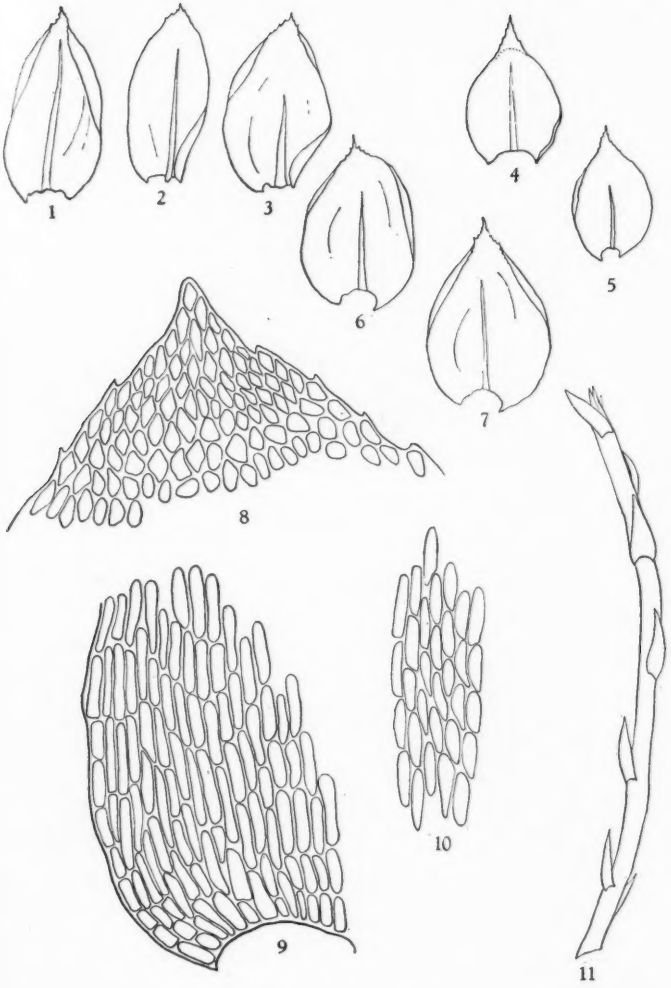
Figs. 1-21. 1-18. *Pireella guatemalensis* Bart. var. *guatemalensis*.—1, 2. Stipe leaves, ×35; 3. Habit sketch, ×4; 4, 5. Branch leaves, ×35; 6-10. Secondary stem leaves, ×35; 11-13. Perichaetial leaves, ×35; 14. Sporophyte showing the scabrous seta, ×14; 15. Capsule, ×16; 16. Median cells, ×1125; 17. Cells of the apical region of the leaf, ×1125; Alar region, ×1125 (figs. 1-18, from the type); 19-21. *Pireella guatemalensis* Bart. var. *mexicana* Arzeni.—19. Habit sketch, ×5; 20. Capsule, ×16; 21. Sporophyte showing the long smooth seta, ×14 (figs. 19-21, from the type).

PLATE 22



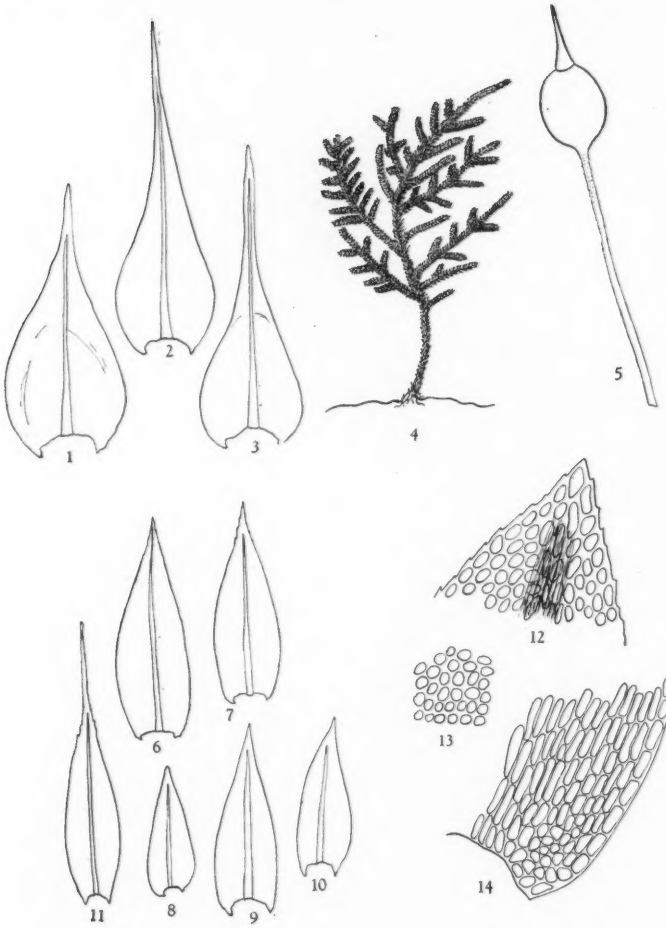
Pireella cavifolia (Card.) Card.—Habit sketch, ×12 (from the type)

PLATE 23



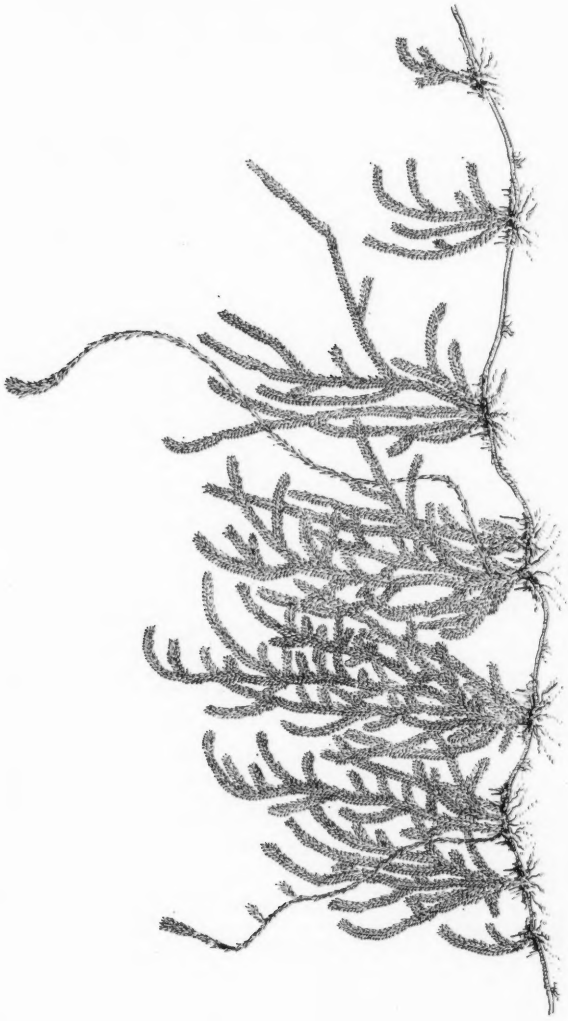
Figs. 1-11. *Pireella cavifolia* (Card.) Card.—1, 2. Secondary stem leaves, ×35; 3-5. Branch leaves, ×35; 6, 7. Stipe leaves, ×35; 8. Leaf apex, and cells of the apical region, ×675; 9. Alar region, ×675; 10. Median cells, ×675; 11. Flagelliform branch, ×675 (figs. 1-10, from the type).

PLATE 24



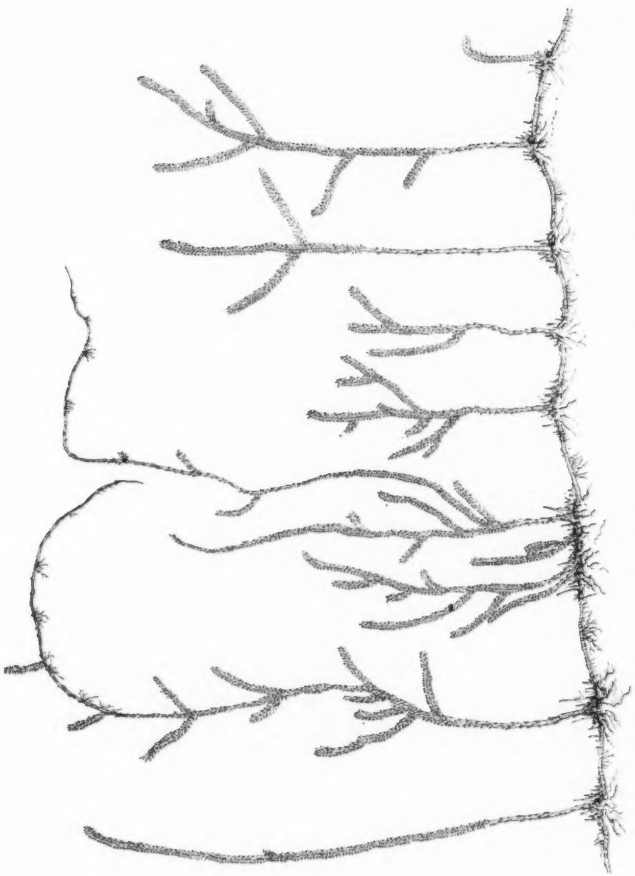
Figs. 1-14. *Pireella mariae* (Card.) Card.—1-3. Secondary stem leaves, ×40; 4. Habit sketch, ×6; 5. Sporophyte showing subglobose capsule and scabrous seta above, ×16; 6, 7. Stipe leaves, ×40; 8-10. Branch leaves, ×40; 11. Perichaetial leaf, ×40; 12. Leaf apex, and cells of the apical region, ×675; 13. Median cells, ×675; 14. Alar region, ×675 (figs. 1-14, from the type).

PLATE 25



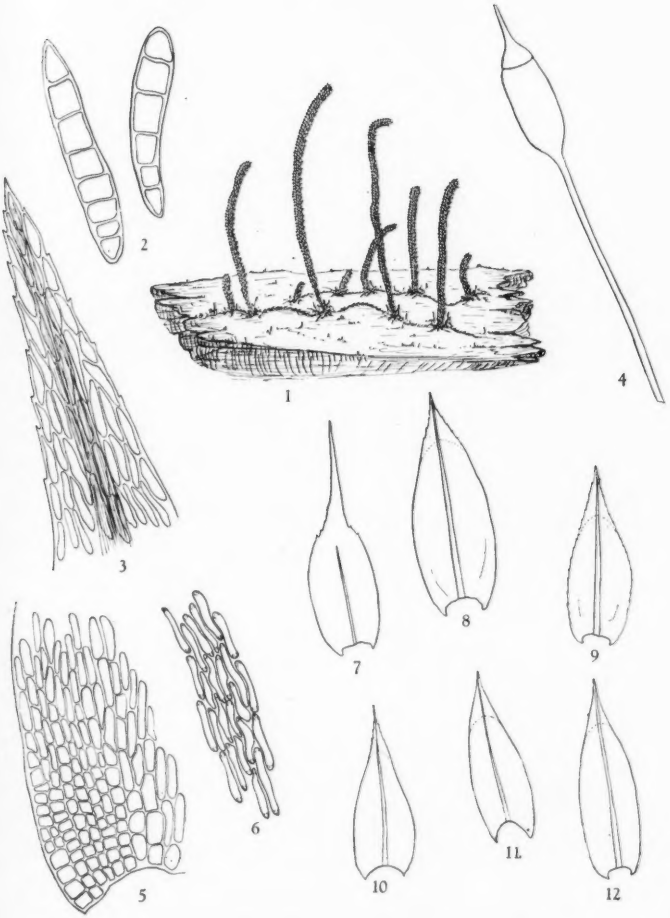
Pireella cymbifolia (Sull.) Card.—Habit sketch showing tufted plants typical of this species, ×12 (from the type, in part)

PLATE 26



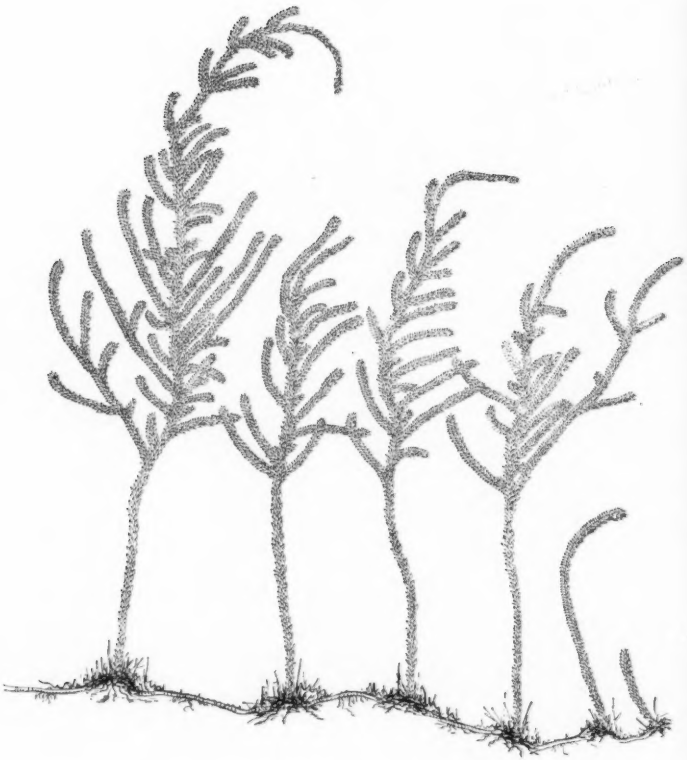
Pirella cymbifolia (Sull.) Card.—Habit sketch showing scraggly, dendroid plants occasionally found in this species, ×12

PLATE 27



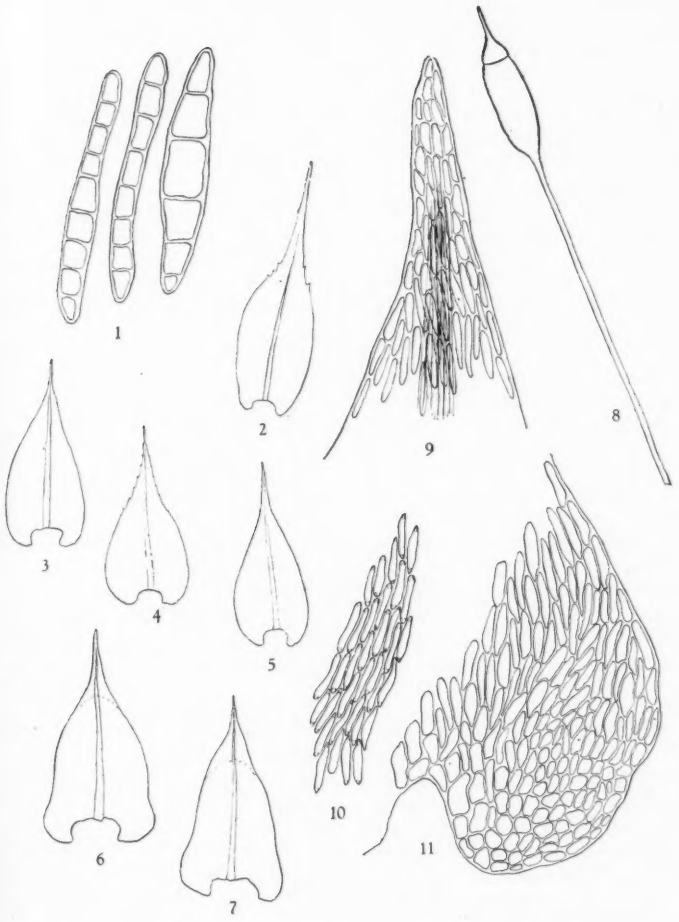
Figs. 1-12. *Pireella cymbifolia* (Sull.) Card.—1. Habit sketch of several dwarf unbranched plants, ×16; 2. Gemmae, ×675; 3. Leaf apex, and cells of the apical region, ×675; 4. Sporophyte, ×20; 5. Alar region, ×675; 6. Median cells showing a single papilla at each end, ×675; 7. Perichaetial leaf, ×35; 8, 9. Secondary stem leaves, ×35; 10-12. Branch leaves, ×35 (figs. 3, 5-12, from the type).

PLATE 28



Pireella pohlii (Schwaegr.) Card.—Habit sketch, ×14

PLATE 29



Figs. 1-11. *Pireella pohlii* (Schwaegr.) Card.—1. Gemmae, ×675; 2. Perichaetial leaf, ×35; 3-5, Branch leaves, ×35; 6, 7. Secondary stem leaves, ×35; 8. Sporophyte, ×20; 9. Leaf apex, and cells of the apical region, ×675; 10. Median cells showing a single papilla at each end, ×675; 11. Alar region, ×675.

Liverworts of Georgia—An Annotated List

Don L. Jacobs
University of Georgia, Athens

The portion of the North American continent politically circumscribed as the State of Georgia includes a geographically and floristically diverse and interesting area. This may, obviously, be said about many political units since few of them are geographically homogeneous, or completely enclosed by natural boundaries. Nevertheless, certain unique features exist which ought to stimulate biotic studies in this State. It is thus somewhat surprising to note the great vacuum which exists in our knowledge of the biota of the region. A few of the factors which may have accounted for this void in the past are: 1) the dearth of qualified personnel to carry on research, 2) insufficient time and recognition given to research, and 3) the richness of the flora and fauna, making the task a large one. It is therefore to be expected that surveys, check lists, and reports of new species will continue to issue from Georgia for some time.

It is to fill partially this vacuum that the present list is published. It is among several that have long been needed and urgently requested by various workers. To my knowledge, no such list has ever been published for Georgia. This work is known to be incomplete; I have been unable to assign names to a number of my collections, and several species have ranges that should include Georgia but have not yet been found. The most notable additions to the list will probably come from the Lejeuneaceae of South Georgia and the coastal islands, and from previously undescribed species.

Most bryophyte collecting in Georgia has been accomplished by transients and reported as partial lists for given localities or as descriptions of new species. Apparently no resident scientists have surveyed the bryophytes of the state. Margaret Brown published the first notable local list of Georgia hepatics in 1924 after a visit to Thomasville, Thomas County, in the Lower Coastal Plain, near the Florida border. Her list of 41 species included 20 that had not been previously reported from the state and, as might be expected, most of them were Floridian species. Some of the same hepatics as well as a few new records were later discovered by Grout (1941) on a winter trip into the great Okefenokee Swamp. He found only 12 liverwort species on his cold and unpleasant trip. Miss Parker (1941) reported 19 species from the Piedmont region just south of the Blue Ridge Mountains. The most exhaustive local survey in the state was made by Gladys Carroll (1945) in the Appalachian (Cumberland) Plateau of extreme Northwest Georgia where she found 45 species of hepatics and 93 species of mosses. Her list included 22 liverworts that had not been previously reported from the state. Most other records are from isolated collections bringing the number to 86 species and 4 varieties. It is interesting to note that despite the myriad collections of plants made by J. K. Small in Georgia, few if any liverworts were included. This is rather surprising in view of the publication of a list of mosses collected by him on Stone Mountain (Schornherst, 1945) as well as other scattered collection references. Among the pioneer bryologists who collected in Georgia were Leo Lesquereux, William Sullivant, and Beyrich. Blomquist has recently published several interesting records.

The present list includes 36 species not reported by other workers. These are marked with an asterisk (*) before the name. While this list is obviously incomplete, it is deemed worthy of publication at this time for several reasons: 1) Time devoted to the project will have to be reduced; 2) additions are being made less frequently; 3) compilation of scattered data should simplify the task of other workers in evaluating their collections and thus result in further reports; and 4) many individuals have requested its early release for reference, range data, etc.

Despite its incompleteness, the present work compares favorably with similar lists. Miss Ammons (1940) recorded 111 species of hepatics from 56 genera for West Virginia, Sharp's list for Eastern Tennessee (1939) contains 114 liverworts, and Patterson (1949) lists 113 species for Virginia. The present Georgia list includes 126 entries.

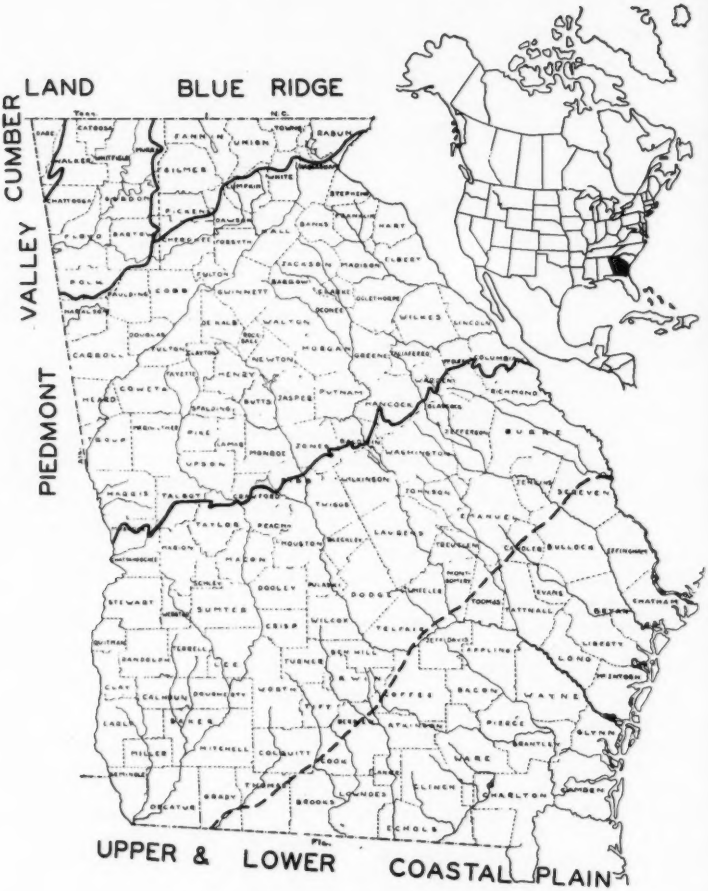
Names and descriptions of at least seven species of liverworts are based on specimens collected in Georgia. Several of the species found in the state are known from only one or two localities, and a few are remarkable examples of split ranges. *Riccia campbelliana*, for example, is known only from California, Texas to Arkansas (Jacobs, 1951), and one station in Georgia.

The arrangement of entries in the following list follows the pattern used by Evans (1940), although in a few cases different synonyms are used. No synonymy is included as this is exhaustively dealt with in Frye and Clark (1937-1947) and other manuals. As far as possible, published range records were traced to their sources and specimens checked in questionable cases. A few errors were disclosed, and these species have been omitted from the list. My own collections which were made chiefly in 1949 and 1950 included 95 of the 126 liverworts listed.

Since county records are of little interest to workers beyond the borders of a state, especially when these political units are as numerous as they are in Georgia, I have made annotations on the basis of the geologic provinces in which each species has been found together with the collectors' names. My collections are indicated by the initials, DLJ. In those species that are being reported for the first time, my collection data and numbers are included.

The accompanying map shows the distribution of the geologic provinces of Georgia. As would be expected, a few species range widely through the state, being found in suitable habitats in every province, whereas others apparently occur only in very restricted habitats in one or two provinces. The Lejeuneaceae are present in greatest profusion in the Lower Coastal Plain, and a number of aberrant forms have been encountered creating a complex taxonomic problem. Some members of even this family are statewide in distribution. Most of the habitats of the state afford only acid substrata, but alkaline conditions occur in the limestone valleys of the Valley and Ridge Province and the lime sinks of the Upper Coastal Plain.

The aquatic Riccias are dealt with in the accompanying list according to the analysis of Lorbeer as published by K. Mueller (1940). The European members of this complex are placed in four species by Lorbeer. Only *R. duplex* Lorbeer is not yet reported from America. *R. canaliculata* Hoffmann, *R. fluitans* L., and *R. rhenana* Lorbeer are included in the present list. Material from California designated as *R. canaliculata* by Carter (1935) agrees well with Lorbeer's analysis but Miss Carter's *R. huebeneriana* Lindenberg agrees best with Lorbeer's *R. rhenana*. Material included under the last name



Geologic provinces of Georgia

in the present list was reported on and described previously by the author (Jacobs, 1950). It should be pointed out that this material seems more robust than that described by Lorbeer but otherwise it matches well including the chromosome number of 16. Possible distinctness of Georgia material could only be discovered by comparative experimental cultures including European material.

The present work would probably not have come to fruition without a Carnegie Foundation Research Grant-in-Aid that furnished funds for extensive travel throughout the state in conjunction with a duckweed survey project.

LIVERWORTS KNOWN TO OCCUR IN GEORGIA

Ptilidiaceae.—Trichocolea tomentella (Ehrh.) Dum.—*Cumberland,* Carrol.

Lepidoziaceae.—Bazzania trilobata (L.) S. F. Gray.—*Cumberland,* Carrol. *Lower Coastal Plain,* DLJ. Lepidozia sylvatica Evans.—*Cumberland,* Carrol; *Lower Coastal Plain,* Brown, DLJ; *Piedmont* and *Blue Ridge,* DLJ. Telaranea nematodes (Aust.) Howe. —*Lower Coastal Plain,* Brown.

Calypogeiaceae.—Calypogeia arguata var. sullivantii (Aust.) F. & C.—*Lower Coastal Plain,* Brown. *C. fissa* (L.) Raddi.—*Cumberland,* Carrol; *Piedmont* and *Coastal Plain,* DLJ. C. nessiana (Massal. & Carest.) K. Muell.—*Cumberland,* Carroll. C. trichomanis (L.) Corda.—*Cumberland,* Carrol; *Piedmont,* Parker, DLJ; *Coastal Plain,* Brown, DLJ; *Blue Ridge,* DLJ.

Cephaloziaceae.—Cephalozia bicuspidata (L.) Dum.—*Cumberland,* Carrol. C. catenulata (Hueben.) Spruce.—*Lower Coastal Plain,* Brown, *Cumberland,* Carrol; Okefenokee Swamp, Group; *Piedmont,* DLJ. C. connivens (Dicks.) Lindb.—*Coastal Plain,* Brown, DLJ; *Piedmont,* Parker, DLJ. C. media Lindb.—*Cumberland,* Carrol; Okefenokee, Grout. Nowellia curvifolia (Dicks.) Mitt.—*Cumberland,* Carrol; *Blue Ridge,* DLJ. Odontoschisma denudatum (Mart.) Dum.—*Cumberland,* Carrol; *Piedmont,* Parker, DLJ. O. prostratum (Sw.) Trev.—*Cumberland,* Carrol; *Blue Ridge* and *Piedmont,* DLJ; *Coastal Plain,* Brown, DLJ.

Cephaloziellaceae.—Cephaloziella hyalina Douin.—*Lower Coastal Plain,* Brown.

Harpanthaceae.—Lophocolea bidentata (L.) Dum.—*Cumberland,* Carrol; *Valley* and *Ridge,* DLJ, *L. cuspidata Limpr.—White Co., Moist humus bank in rich woods, 2½ mi. south of Unicoi Gap, April 14, 1949, DLJ 849. Whitfield Co., Moist rock at Freeman Springs, November 13, 1949, DLJ 1104. L. heterophylla (Schrad.) Dum.—*Cumberland,* Carrol; *Coastal Plain,* Brown, DLJ; *Piedmont,* DLJ. *Chiloscyphus polyanthus (L.) Corda.—Jenkins Co., Decaying log in Dukes Pond, May 21, 1949, DLJ 930.

Jungermanniaceae.—*Isopaches bicrenatus (Schmid.) Buch.—Harris Co., Sandy clay trail in moist woods on Pine Mountain, July 28, 1949, DLJ 1006. Jungermannia lanceolata Schrad.—*Cumberland,* Carrol. Nardia geoscyphus (DeNot.) Lindb.—"Ga." in Underwood's catalogue N. A. Hep., Bull. Ill. St. Lab. N. H. No records. N. lescurii (Aust.) Underw.—Rabun Co., Wet rocks, moist soil, Tallulah Falls; 1859, Lesquereux. Rabun Co., Underwood 389. Plectocolea crenulata (Smith) Buch.—*Piedmont,* Parker, DLJ; *Lower Coastal Plain,* Brown. P. crenulata var. gracillima (Hook.) F. & G.—*Lower Coastal Plain,* Brown.

Marsupellaceae.—*Marsupella emarginata (Ehrh.) Dum.—Rabun Co., Wet granite on shaded face of Glade Mtn., April 17, 1949, DLJ 888. M. sphacelata (Gieseke) Dum. —*Blue Ridge,* DLJ, Small. M. sphacelata var. erythrorhiza (Limpr.) Schiffn.—"Ga." in Evans and Nichols, Bryophytes of Connecticut, 1908.

Plagiochilaceae.—Plagiochila asplenioides (L.) Dum.—*Cumberland,* Carrol; *Piedmont,* Parker, DLJ. *P. floridana Evans.—White Co., Moist rock on wooded slope, 2½ mi. south of Unicoi Gap, April 14, 1949, DLJ 846. P. ludoviciana Sull.—*Cumberland,* Carrol; *Lower Coastal Plain,* Brown, Okefenokee, Grout. *P. smallii Evans.—Decatur Co., Moist bank of Flint R., 7 miles north of Bainbridge, August 3, 1949, DLJ 1013. *P. austini Evans.—Upson Co., On beech trunk along Flint R., 4 miles southeast of Woodbury, August 20, 1949, DLJ 1046. P. sullivantii Goetsche.—"Ga." Ammons manual, p. 68, 1940. P. undata Sull.—*Cumberland,* Carrol; *Valley & Ridge* and *Piedmont,* DLJ.

Scapaniaceae.—*Diplophyllum andrewsii Evans.—Rabun Co., Wet bark on tree base in rich cove, ½ mi. southeast of Glade Mtn., DLJ 866. D. apiculatum (Evans) Steph. —*Cumberland,* Carroll; *Piedmont,* Parker, DLJ; *Blue Ridge,* DLJ. Scapania nemorosa (L.) Dum.—Suitable habitat throughout. S. undulata (L.) Dum.—*Cumberland,* Carrol; *Piedmont,* DLJ.

Porellaceae.—Porella pinnata L.—*Cumberland,* Carrol; *Lower Coastal Plain,* Brown; *Piedmont,* DLJ. P. platyphylla (L.).—*Piedmont,* Cobb Co., McFarlin 1077. P. Platyphylla var. platyphylloidea (Schwein.) F. & C.—*Cumberland,* Carrol; *Piedmont,* Parker, DLJ; *Blue Ridge,* DLJ, McVaugh; *Valley & Ridge,* DLJ.

Radulaceae.—Radula andicola Steph.—*Cumberland,* Carrol. R. australis Aust.— "Georgia & Florida," W. C. Barbour, The Bryol. 5:93. 1902. R. complanata (L.) Dum. —*Cumberland,* Carrol; *Lower Coastal Plain,* Brown; *Piedmont,* DLJ, Parker; *Blue Ridge,* DLJ. R. obconica Sull.—*Cumberland,* Carrol; *Piedmont,* DLJ. R. sullivantii Aust.— *Blue Ridge,* Sullivant, Lesquereux; *Piedmont* and *Blue Ridge,* DLJ.

Frullaniaceae.—Frullania asagrayana Mont.—*Cumberland,* Carrol; *Piedmont,* Parker, DLJ; *Blue Ridge,* DLJ. *F. brittoniae Evans.—Greene Co., Tree trunk at Watson's Spring, 12 mi. southeast of Watkinsville, May 1, 1949, DLJ 898. F. eboracensis Gottsche. —*Cumberland,* Carrol; *Piedmont,* DLJ, Parker; *Valley & Ridge,* DLJ. F. inflata Gottsche. —*Cumberland,* Carrol; *Upper Coastal Plain,* R. Harper, DLJ. F. kunzei Lehm. & Lindenb.—*Lower Coastal Plain,* Brown, Grout, DLJ. F. obcordata Lehm. & Lindenb.— *Lower Coastal Plain,* Brown. *F. plana Sull.—Clarke Co., Boulder in moist woods at edge of U. of Ga. campus, November 12, 1948, DLJ 713. Oglethorpe Co., Rock on wooded bank; DLJ 762. *F. riparia Hampe.—Clarke Co., Tree trunks on U. of Ga. campus, April 7, 1949, DLJ 805. Greene Co., Tree trunks; DLJ 900. F. squarrosa (Bl.R.N.) Dum.—*Cumberland,* Carrol; *Piedmont,* DLJ, Parker; *Coastal Plain,* Brown, DLJ, Okefenokee, Grout. Jubula pennsylvanica (Steph.) Evans.—*Cumberland,* Carrol; *Blue Ridge* and *Piedmont,* DLJ.

Lejeuneaceae.—Mastigolejeunea auriculata (Wils. & Hook.) Schiffn.—*Lower Coastal Plain,* Brown, Grout (Okefenokee Swamp). Leucolejeunea clypeata (Schwein.) Evans.— *Cumberland,* Carrol; *Piedmont,* Parker, DLJ; *Blue Ridge & Coastal Plain,* DLJ. L. conchifolia Evans.—*Lower Coastal Plain,* Brown. *L. unciloba* (Lindenb.) Evans.—*Cumberland,* Carrol; *Piedmont & Blue Ridge,* DLJ; *Lower Coastal Plain,* Brown, Grout (Okefenokee); *Piedmont* and *Blue Ridge,* DLJ. Euosmolejeunea duriuscula (Nees.) Evans.— *Lower Coastal Plain,* Brown. E. parvula Evans.—*Lower Coastal Plain,* Brown. Rectolejeunea maxonii Evans.—*Lower Coastal Plain,* Brown; *Piedmont* and *Upper Coastal Plain,* DLJ. *Harpalejeunea ovata (Hook.) Schiffn.—"Va. to N. Ga." Ammons manual p. 85. 1940. No records.—White Co., Moist rock on wooded bank, 2½ mi. south of Unicoi Gap, April 14, 1949, DLJ 837.—Rabun Co., Tree trunk; DLJ 867, 881. *Lejeunea cavifolia (Ehrh.) Lindb.—Bulloch Co., Base of tree in moist hammock 6 mi. southeast of Statesboro, April 3, 1949, DLJ 797. Upson Co., Trunk of beech tree, DLJ 1047. L. flava (Sw.) Nees.—*Coastal Plain,* Brown, DLJ. *L. floridana Evans.—Glynn Co., Tree trunk, Jekyll Island, St. Simon Island, June 21, 22, 1949, DLJ 952, 959. Upson Co., Sweet gum trunk, DLJ 1048. Whitfield Co., Moist rocks, DLJ 1105. *L. minutiloba Evans.—Decatur Co., Moist river bank of Flint R., 7 mi. north of Bainbridge, August 3, 1949, DLJ 1014. L. patens Lindb.—*Blue Ridge,* Parker, DLJ. Microlejeunea bullata (Tayl.) Evans.—Okefenokee, Grout; *Piedmont,* DLJ. M. laetevirens (Nees & Mont.) Evans.—*Cumberland,* Carrol; *Lower Coastal Plain,* Brown; *Piedmont & Blue Ridge,* DLJ. *M. ruthii Evans.—White Co., Moist rocks and base of tree in rich cove, 2½ mi. south of Unicoi Gap, April 14, 1949, DLJ 837, 850. Greene Co., Tree trunk, DLJ 902. M. ulicina (Tayl.) Evans.—*Cumberland,* Carrol; *Piedmont,* DLJ. Cololejeunea biddlecomiae (Aust.) Evans.—*Lower Coastal Plain,* Brown; *Piedmont,* DLJ. C. contractiloba Evans.—Okefenokee, Grout. Leptocolea cardiocarpa (Mont.) Evans.—*Lower Coastal Plain,* Brown.

Fossombroniaceae.—Fossombronia braziliensis Steph.—*Lower Coastal Plain,* Brown. *F. cristula Aust.—Oglethorpe Co., Sandy clay in old road along Millstone Creek, 12 mi. north of Lexington, July 20, 1949, DLJ 984. Baker Co., Pond margin, DLJ 1011. Gordon Co., Beside springs, DLJ 1079, 1098. Upson Co., Old road DLJ, 1049. Floyd Co., Old road, DLJ 1086, 1093. *F. foveolata Lindb.—Shallow soil associated with seepage paths at upper edge of most granite outcrops examined in the Piedmont of Georgia.—Barrow Co., DLJ 1061; Clarke Co., DLJ 757, 771; Gwinnett Co., DLJ 775; Heard Co., DLJ 1005; Madison Co., DLJ 1066; Oglethorpe Co., DLJ 820; Rockdale Co., DLJ 913; Walton Co., DLJ 778. *F. wondracseckii (Corda) Dum.—Spalding Co., Wet, partially shaded, rocky bank of Cabin Creek, 3 mi. northeast of Griffin, August 20, 1949, DLJ 1050.

Pelliaceae.—Pellia epiphylla (L.) Corda.—*Piedmont,* Parker, DLJ; *Blue Ridge,* DLJ. P. neesiana (Gottsche) Limpr.—*Cumberland,* Carrol; *Valley Ridge,* DLJ; *Piedmont,* Parker.

Pallaviciniaceae.—Pallavicinia lyellii (Hook.) S. F. Gray.—Numerous stations throughout state but most abundant in *Lower Coastal Plain.*

Metzgeriaceae.—Metzgeria ciliifera Schweinitz.—*Lower Coastal Plain,* Brown. M. conjugata Lindb.—*Piedmont,* Parker, DLJ. *M. crassipilis (Lindb.) Evans.—White Co., Wet rocks in rich cove, 2½ mi. south of Unicoi Gap, April 14, 1949, DLJ 836. Towns Co., Base of tree, DLJ 853. Rabun Co., Base of tree, bark, DLJ 881. M. furcata (L.) Dum.—*Cumberland,* Carrol; *Piedmont,* Parker, DLJ.

Riccardiaceae.—Riccardia latifrons Lindb.—*Cumberland,* Carroll; *Coatsal Plain,* Brown,

Grout (Okefenokee), DLJ; *Blue Ridge,* DLJ. R. multifida (L.) S. F. Gray.—*Cumberland,* Carrol; *Blue Ridge,* A. J. Sharp, W. H. Duncan; *Piedmont,* DLJ. R. pinguis (L.) S. F. Gray.—*Cumberland,* Carrol. *R. sinuata (Dicks.) Trev.—Clarke Co., Roots & soil in erosion gully in White Hall Forest, December 11, 1948, DLJ 732, 758.

Marchantiaceae.—Marchantia domingensis Lehm. & Lindenb.—"Ga." in Evans, Trans. Conn. Ac. A. & S. 21: 201-213. 1917. Clarke Co., Vertical stream bank, DLJ 773. Whitfield Co., Vertical stream bank, DLJ 1101. *M. polymorpha L.—Gordon Co., Bank of clear stream, 3 mi. northwest of Sugar Valley, June 23, 1950, DLJ 1167; Clarke Co., Bank of Stream on Univ. of Ga. Campus, June 20, 1951, DLJ 1177. Conocephalum conicum (L.) Dum.—*Cumberland,* Carrol; *Blue Ridge, Valley & Ridge, Piedmont,* DLJ. *Lunularia cruciata (L.) Dum.—Fulton Co., Under benches in commercial greenhouse in Atlanta, July 31, 1951, DLJ 1178. Dumortiera hirsuta (Sw.) Nees.—*Cumberland,* Carrol; *Blue Ridge,* W. H. Duncan; *Piedmont, Valley & Ridge,* DLJ. D. *hirsuta* var. nepalensis (Tayl.) F. & C.—*Coastal Plain,* Decatur Co., R. M. Harper 1193a.

Rebouliaceae.—Reboulia hemisphaerica (L.) Raddi.—*Cumberland,* Carrol; *Lower Coastal Plain,* Brown; *Piedmont,* DLJ. Mannia fragrans (Balbis) F. & C.—*Piedmont,* Rogers McVaugh & Pyron. Asterella tenella (L.) Beauv.—*Lower Coastal Plain,* Brown; *Piedmont,* Parker, DLJ; *Valley & Ridge,* DLJ.

Ricciaceae.—Ricciocarpus natans (L.) Corda.—Bryan Co., Edge of swamp, 2 mi. south of Richmond Hills, June 19, 1949, DLJ 946. Decatur Co., DLJ 1022. Baker Co., DLJ 1030. McIntosh Co., Sapelo Island, DLJ 1139. *Riccia austini Steph.—Walton Co., Shallow soil on granite outcrops at Loganville, March 27, 1949, DLJ 781. Found on all other granite outcrops examined: Clarke, De Kalb, Heard, Oconee, Oglethorpe, Madison, Rockdale, and Spalding Counties, DLJ 825, 895, 906, 910, 1003, 1053, 1062, & 1108. R. beyrichiana Hampe.—Jackson Co., "Between Jefferosn & Gainsville," Beyrich, August 13, 1833. Most granite outcrops in the Piedmont, DLJ 907, 911, 915, 980, 1001, 1063. R. bifurca Hoffm.—*Piedmont,* McVaugh, DLJ; *Coastal Plain* and *Valley* and *Ridge,* DLJ. *R. campbelliana Howe.—Oglethorpe Co., Herb mat on granite at Echol's Mill, 12 mi, north of Lexington, April 10, 1949, DLJ 826, 917. *R. canaliculata Hoffmann.—Baker Co., Springfield & Mossy Ponds; Crisp Co., Gum Creek spring; Sapelo Island, DLJ 1010, 1031, 1036, 1138 respectively. R. dictyospora Howe.—*Piedmont,* all granite outcrops, McVaugh, DLJ. R. donnellii Aust.—*Piedmont,* McVaugh, DLJ; *Lower Coastal Plain* (Okefenokee Swamp), Grout. *R. eldeeniae Jacobs.—*Piedmont,* Clarke and Oglethorpe counties, on granite outcrops, DLJ 908, 920, 975, and 978, (see Jacobs, 1950). *R. fluitans L.—Decatur Co., Hog wallows & woodland ponds 5 mi. northeast of Bainbridge, DLJ 1022a. R. hirta (Aust.) Underw.—*Lower Coastal Plain,* Brown; *Piedmont,* all granite outcrops, DLJ. *R. membranacea Gottsche & Lindenb.—Oglethorpe Co., Mud in road through woods along Millstone Creek, 12 mi. north of Lexington, July 20, 1949, DLJ 983. *R. rhenana Lorbeer.—Harris Co., Blue Springs on Callaway Estate; Gordon Co., Blackwood Springs, Johnson's Spring; Bartow Co., Water Cress Springs, 2½ mi. northeast of Adairsville, DLJ 988 & 989, 1076, 1096, 1081, & 1082 respectively. *R. sorocarpa Bisch.—Clarke Co., Along railroad near Tan Yard Branch, Athens; (not reported) Jan. 4, 1914, Howe. Jenkins Co., Sandy clay path, Duke's Pond, DLJ 1056; Madison Co., Granite outcrop, DLJ 1065. *R. sullivantii Aust.—Oglethorpe Co., Wet shaded margin of granite at Echol's Mill, 12 mi. north of Lexington, May 16, 1949, DLJ 919; Walton Co., Wet shaded margin of granite, DLJ 963; Baker Co., Muddy pond margin, DLJ 1009; Floyd Co., DLJ 1083, 1092. *R. tenella Jacobs.—Gwinnett Co., Shallow depression in granite, DLJ 783; Rockdale Co., Shallow soil on granite, DLJ 916; Walton Co., Shallow soil on granite, DLJ 964; De Kalb Co., Stone Mtn., DLJ 1109. See Jacobs, 1950.

Sphaerocarpaceae.—Sphaerocarpus donnellii Aust.—Reported from Georgia in: Allen, C. E. 1934. S. texanus Aust.—*Lower Coastal Plain,* Brown; *Piedmont,* DLJ.

Anthocerotaceae.—Anthoceros carolinianus Michx.—*Cumberland,* Carrol; *Lower Coastal Plain,* Brown; *Piedmont,* DLJ. *A. crispulus (Mont.) Douin.—Oglethorpe Co., Margin of granite, moist soil at Echol's Mill, 12 mi. north of Lexington, April 10, 1949, DLJ 817. A. laevis L.—*Cumberland,* Carrol; *Valley* and *Ridge,* DLJ. A. punctatus L.—*Lower Coastal Plain,* Brown; *Piedmont,* DLJ. A. ravenelii Aust.—*Lower Coastal Plain,* Brown. *Notothylas orbicularis (Schweinitz) Sull.—Gordon Co., Bank of Blackwood Springs, November 12, 1949, DLJ 1078; Floyd Co., DLJ 1087, 1091.

REFERENCES

ALLEN, C. E. 1934—A diploid female gametophyte of Sphaerocarpos. Proc. Nat. Acad. Sci. 20:147-150.

AMMONS, NELLE 1940—The liverworts of West Virginia. Amer. Midl. Nat. 23:3-164.

BLOMQUIST, H. L. 1939—Notes on southern hepaticae. The Bryologist 42:31.

BROWN, M. S. 1924—Hepatics in Georgia. Ibid. 27:31-34.

CARROLL, GLADYS 1945—The Bryophytes of the Appalachian Plateau in northern Georgia. Ibid. 48:11-24.

CARTER, A. M. 1935—Riccia fluitans L.—a composite species. Bull. Torr. Bot. Club 62:33-42.

EVANS, A. W. 1940—List of hepaticae found in the United States, Canada, and Arctic America. The Bryologist 43:133-138.

FRYE, T. C. AND LOIS CLARK 1937-47—Hepaticae of North America. Nos. 1-5. Univ. Wash, Publ. Biol. 6:1-1018.

GROUT, A. J. 1941—Bryology in the Okefenokee. The Bryologist 44:120-122.

JACOBS, D. L. 1949—Two new Riccias from Georgia. Ibid. 52:167-172.

———1950—Economic potential of a new aquatic liverwort. Bull. Ecol. Soc. 31:57.

———1951—Ricciaceae of Arkansas. The Bryologist 54:

PARKER, DOROTHY 1941—Some hepaticae from northeastern Georgia. Ibid. 44:154.

PATTERSON, P. M. 1949—The bryophytes of Virginia, I—Bryophytes reported in the literature. Castanea 14:1-49.

SCHORNHERST, R. O. 1945—Mosses of Stone Mountain, Georgia. The Bryologist 48:29-33.

SHARP, A. J. 1939—Taxonomic and ecological studies of eastern Tennessee bryophytes. Amer. Midl. Nat. 21:267-354.

Toxic Fresh-water Algae

William Marcus Ingram
Department of Health, Education, and Welfare
U. S. Public Health Service, Ohio-Tennessee Drainage Basins, Cincinnati, Ohio

G. W. Prescott
Michigan State College, East Lansing

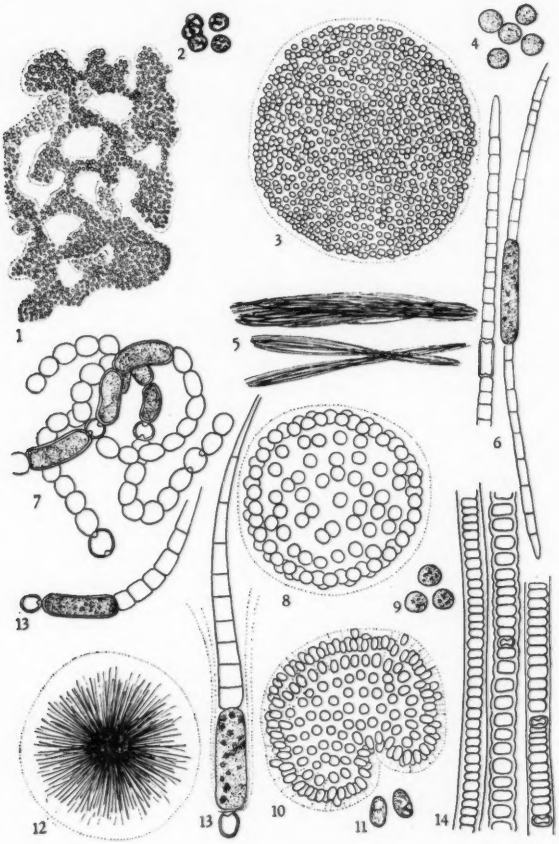
More and more interest is being aroused in the toxic effects of fresh-water algae on animals, partly because of a recent upswing in the number of reported cases of animal poisoning related to algal blooms. Another source of interest stems from a growing appreciation of the strictly scientific and biological problems involving the physiology of algae, especially those which produce toxic substances (possibly toxins), antibiotics, and growth-stimulating excretions. The present paper is intended as a contribution toward a summary of our present knowledge of toxic fresh-water algae. Especially it is predicated on interest that was aroused in the presentation of a lecture by the senior author, *Organisms Encountered in Water Supplies*. This was presented to classes in Advanced Sanitary Engineering Training in Water Pollution Investigations, and in Advanced Training for Bacteriologists Concerned with Water Analysis conducted at the Environmental Health Center, Public Health Service, Cincinnati, Ohio.

Herein, we shall outline in a general way the effects of toxic fresh-water algae on man, domestic animals, and fish for especially those workers who are concerned with water pollution control programs. Thorough quantitative data are not available on toxic fresh-water algae as they are related to illness or death among fish and higher forms of life. It is the hope of the authors that through such a discussion of toxic algae more quantitative data will be brought into the literature from those who are concerned with water supplies as they may relate to man, domestic animals, and to fish and game. Only additional data on specific cases of poisoning by particular algae can present a comprehensive picture for the United States, thus permitting such information to be analyzed critically in the future.

The algae that have been reported as causing death and, or, illness in mammals, birds, and fish through toxic action are, as far as we know, the blue-greens, Cyanophyta. The Cyanophyta can be differentiated from other groups of algae primarily by the fact that their pigments are not contained in chromatophores (pigment bodies). Unlike all other algae the pigments are in solution and are more or less confined to the peripheral portion of the protoplast. Included are chlorophyll-a, carotenes, distinctive xanthophylls, a blue pigment, c-phycocyanin, and a red pigment—c-phycoerythrin. The blue-green algae possess a primitive type of nucleus, the central body, which lacks a nuclear membrane and a nucleolus, in which respect they are similar to bacteria.

The genera of the Cyanophyta that appear to possess toxic species are: *Microcystis* (figs. 1-4),* *Anabaena* (fig. 7), *Aphanizomenon* (figs. 5-6) *Nod-*

* See Daily (1942), Amer. Midl. Nat. 27(3):636-661 for remarks on *Microcystis flos-aquae;* also Smith (1950), Fresh-water Algae of the United States, p. 556, *Microcystis* transferred to *Polycystis*, and Prescott (1951), Algae of the Western Great Lakes Area, p. 455 for remarks on *Microcystis* and *Polycystis*.



Figs. 1-14.—1. *Microcystis aeruginosa* Kütz. A colony of cells showing clathrate and perforate gelatinous sheath inclosing numerous, irregularly arranged individuals. 2. *Microcystis aeruginosa* Kütz. Individual cells showing the false vacuoles which refract the light and give the colony a "blackish" appearance when viewed microscopically. 3. *Microcystis flos-aquae* (Wittr.) Kirch. A globular colony. 4. *Microcystis flos-aquae* (Wittr.) Kirch. Individual cells with homogeneously granular contents. 5. *Aphanizomenon flos-aquae* (L.) Ralfs. Diagram of flake-like colonies as they appear macroscopically. 6. *Aphanizomenon flos-aquae* (L.) Ralfs. Individual trichomes, showing (right) the enlarged spore and the heterocyst (left) characteristically located near the median region of the plant. 7. *Anabaena flos-aquae* (Lyng.) Bréb. A tangled filament showing the sausage-like spores. 8. *Coelosphaerium Kuetzingianum* Näeg. Colony. 9. *Coelosphaerium Kuetzingianum* Näeg. A few cells showing homogeneously granular content. 10. *Coelosphaerium Naegelianum* Unger. Colony in which radiating fibrils are often visible; the cells crowded at the periphery of the colonial sheath. 11. *Coelosphaerium Naegelianum* Unger. Few cells showing false vacuoles which refract the light and give the colony a brownish or purplish color when viewed microscopically. 12. *Gloeotrichia echinulata* (J. E. Smith) P. Richter.

ularia (fig. 14), *Gloeotrichia* (figs. 12-13), and *Coelosphaerium* (figs. 8-11). It has been demonstrated that not all blue-green algae are toxic to higher organisms (Wheeler et al., 1942).

The majority of the data published on toxic fresh-water algae in relation to cattle and poultry deaths in the United States has appeared in the literature of veterinarian medicine, Cotton (1914), Gillam (1925), Woodcock (1927), Fitch et al. (1934), Deem and Thorp (1939) Quin (1943) and Brandenburg and Shigley (1947). Tisdale (1931, 1931a), Veldee (1931), Wheeler et al. (1942), and Olson (1949, 1952) have commented on human gastroenteritis as possibly involving certain algae. Prescott (1933, 1939, 1948) and Mackenthum et al. (1948) have written in reference to algae that kill fish. Olson (1949) has published a brief summary on the historical aspects of toxic plankton. Scott (1952) has presented a general paper on fresh-water algal toxins.

ACKNOWLEDGMENTS

The writers wish to express their appreciation to the following individuals who made constructive suggestions during the preparation of the manuscript: W. W. Towne, Basin Engineer; J. W. Wiseman, Assistant Basin Engineer; and C. H. Hull, Engineer, all of the Ohio-Tennessee Drainage Basins Office, Division of Water Pollution Control, Public Health Service, and Dr. A. F. Bartsch, Biologist, Division of Water Pollution Control, Public Health Service.

Credit is due Margaret J. Blackham, Secretary, Ohio-Tennessee Drainage Basins Office, who gave of her secretarial skill in manuscript preparation.

HUMAN GASTROENTERITIS AND ALGAE

In consideration of human gastroenteritis as related to algal cells, it can be said that human outbreaks have not been traced directly to the contamination of drinking water by these organisms. It has been suggested in the literature by inference, and also directly, that algal cells may be connected with human gastro-intestinal outbreaks, which are presumably water-borne, when bacteriological evidence cannot be offered to explain them.

After the outbreak of human gastroenteritis involving suspected water supplies that occurred in Charleston, West Virginia, from the latter part of October into early November, 1930, in which between 8,000 and 10,000 persons were affected, Tisdale (1931) made the following comment: "Bacteriological tests indicate that measured by the present accepted U. S. Treasury standards for bacteria counts, the water was safe. Microscopical tests during August and September revealed growths of blue-green algae of several different types capable of causing the objectionable odor. Cycles of algae growths were followed by development of crustacea, daphnia and other allied water flea organisms being present in such great numbers that a glassful of the raw river water appeared to be actively vibrating from the infinite number of organisms present. Whether the intestinal disorders in the city during the latter part of October and in early November can be attributed to the water is still a mystery; but it is a fact that widespread disorders were noted wherever the water supply mains went." Tisdale (1931) commented further by quoting from epidemiological studies made by Public Health Service personnel who were called into the

Diagram of colony in which filaments radiate from a common center, forming tapioca-like bodies suspended in the water. 13. *Gloeotrichia echinulata* (J. E. Smith) P. Richter. Individual plants showing basal heterocyst and spore, from which the vegetative cells taper to a hair-like extremity. 14. *Nodularia spumigena* Mert. Filaments of short, compressed cells.

area, that the character of symptom onset and subsequent symptoms did not suggest bacterial infection. Rather, the symptoms were those that might be exhibited by one who had been poisoned by a substance acting as a gastro-intestinal irritant. The symptoms shown were similar to those which might develop in an individual who had been given a strong cathartic. Tisdale (1931) includes the following statements in his concluding remarks: "Whether products of anaerobic decomposition of nitrogenous or carbonaceous matter could be sufficiently concentrated in the water to produce a condition simulating food poisoning in those drinking the water has not been shown. Neither the U. S. Public Health Service nor the State Health Department [West Virginia] has stated definitely that the bacteriologically safe water supply was responsible for the epidemic, but it is certain that most unusual conditions have existed with reference to intensity of raw water pollution."

Tisdale (1931a) in a comprehensive paper, generally and thoroughly summarized the effects of the 1930-31 drought on public water supply in the drainage basins of the Ohio and Potomac Rivers. Some medical men in Ohio River cities of Ohio proffered two theories for the so-called "intestinal influenza" associated with the 1930-31 drought. One was that a virus caused "intestinal influenza" or human gastroenteritis; the second theory included the thought that a toxic substance was in the river water, produced there by the decomposition of organic substances of both plant and animal origin. From a bacteriological standpoint water was given a clean "bill of health." The following unusual algal conditions are noted in Tisdale's (1931a) article: 1) at Louisville the Ohio River had an algal and plankton content of many thousands per milliliter instead of the usual hundreds; 2) at Charleston, West Virginia, organic matter in the form of algae and protozoans was extremely high, necessitating a 20 lb./m.g. chlorine dosage to supply the chlorine demand; 3) one very notable and persistent condition in all drought states was the prodigious algal growth in storage basins and streams resulting in an accompanying organic load with attendant tastes and odors; 4) intestinal disorders appeared in the following areas during the drought: Washington, D. C.; Charleston, Weston, and Sisterville, West Virginia; Ashland and Louisville, Kentucky; and Portsmouth, Ironton and Cincinnati, Ohio. The idea is put forth at this point in his paper that ". . . it may be that algae in heavy concentrations with the resulting organic load may be a factor in bringing about these troubles (intestinal disorders)."

Veldee (1931), in commenting epidemiologically on suspected water-borne gastroenteritis in reference to the drought period of 1930-1931 in the Ohio River Basin, states in conclusion: "The evidence strongly suggests that the acute gastro-intestinal symptoms were brought about by the presence in the water of some chemical irritant whose physiological action simulated a strong purgative. The evidence does not show whether this chemical irritant was a cleavage product of bacterial action, a new chemical produced by bacterial synthesis, or the result of increased chemical concentration in the water brought about by a decrease in the diluent. There is at least a slight suggestion from the evidence that the toxic substance originated in the Great Kanawha River [in West Virginia] and was released into the Ohio when the dams were lowered on the Great Kanawha late in December, thereby releasing the pent up wastes in concentrated form which affected the public supply of each city as it moves downstream." In reference to wastes that entered a water treatment

plant at Charleston, West Virginia, from the Elk River Pool and the Great Kanawha River, Veldee (1931) lists the following: domestic sewage; soluble wastes from the garbage dump of Charleston, West Virginia; a variety of industrial wastes, and the dissolved products from a heavy sludge layer representing the accumulation of years covered by a dense algal growth.

Wheeler et al. (1942) state that no human outbreaks have ever been traced to algal contamination of drinking water, and that it is probable that the tastes and odors almost invariably associated with great algal pollution would cause human beings to seek other sources for drinking water before enough of the polluted water to be harmful would be consumed. These authors forward a statement involving human allergy when they write: "It should be noted that the presence of algae in drinking water, in addition to causing tastes and odors, may have some importance from the standpoint of the allergist as algae may, on occasion, liberate considerable amounts of protein in water. If allergic reactions to algae proteins do occur, they would be as uncommon as they would be obscure and hardly likely to occur as a public health problem."

Olson (1949) has written stating that public interest in toxic algae is associated with their possible relations to surface water supply problems; this reference generally is to the outbreaks of gastroenteritis along the Potomac and Ohio Rivers during the drought years discussed above. With reference to the opinion by Olson (1949) that the work of Tisdale (1931, 1931a), and Veldee (1931) ascribed the gastro-intestinal upsets to causes that were sometimes vague, the writers wish to emphasize that the thorough engineering and epidemiological studies these men carried on reveal no single specific cause for gastroenteritis that could be pointed to with confidence. Further, Veldee (1931) in conclusion states that the gastroenteritis in the Ohio River Basin epidemiologically is reminiscent of some chemical irritant. He did not, of course, make a complete chemical analysis of the water. Olson (1949) further states: "It is suggested here that the algae-blooms, mentioned prominently in reports of the conditions which existed at the time, may have been at least in part responsible." This suggestion is a theme in Tisdale's (1931a) second paper on gastroenteritis in relation to algae in the Ohio River as described above.

Olson (1952) in a paper on toxic plankton has speculated on human illness as related to toxic fresh-water algae.

TOXICITY OF ALGAE TO DOMESTIC ANIMALS

In contrast to the lack of specific evidence to the causing of illness and death in human beings by fresh-water algae, data are available from field observations to show that certain algae will cause death or illness in domestic animals upon drinking of water fouled by blooms of certain species. Data are also available from laboratory experimental work to show the effects of certain toxic algae on guinea pigs, mice, and poultry.

Beginning in 1878, data have accumulated gradually in the literature which positively relate deaths of domestic animals to the blooming of algae, especially in standing bodies of water. Francis (1878) recorded the first instance of death among domestic animals attributable to algae, noting that the offender was an algal bloom of *Nodularia spumigena* Mert. Francis (1878) made his observations in Lake Alexandrina in South Australia. This alga had accumulated in a scum 2-6 inches thick on the lee shores of Lake Alexandrina where

it was blown by the wind. He observed that the following animals died after having imbibed this water: sheep, after 1 to 6 or 8 hours; horses, after 8 to 24 hours; dogs, after 4 to 5 hours; pigs, after 3 or 4 hours.

The next recorded deaths of domestic animals attributable to toxic algae occurred in 1882, 1883, and 1884, and were reported by Porter (1886), Arthur (1886, 1886a), and Stalker (1886) from Lakes Cordova, Sakatah, and Tetonka at Waterville, Minnesota. In connection with these reports, Arthur (1886a) wrote that the only plausible hypothesis to account for the death of animals, largely cattle, that could be advanced was the possession by algae of ". . . some toxic or other baneful properties sufficiently powerful to kill a cow in a half hour or more after drinking freely of it." The alga that was responsible for the cattle deaths in the Waterville area has been called variously, *Rivularia fluitans* Cohn, *Gloeotrichia Pisum* (Ag.) *Thur.*, and *Gloeotrichia echinulata* (J. E. Smith) Richter (See Fitch et al., 1934). As in the report by Francis (1878) of the cattle killed in Australia, the wind had blown algae together, concentrating them and forming a scum on the water when the deaths occurred. Stalker (1886) referring to the same incident wrote that no losses of cattle occurred after the odor from the above mentioned lakes became offensive.

Nelson (1903-04) reported cattle killed by algal blooms in one of the shallow lakes in depressions of Fergus Falls moraine in Minnesota. The alga responsible for these deaths was *Aphanizomenon flos-aquae* (L.) Ralfs. When cattle were fenced away from the source of water where this alga was blooming none showed poison symptoms.

From 1918 to 1934 the Division of Veterinary Medicine of the University of Minnesota had occasion to investigate several outbreaks of algal poisoning as reported by Fitch et al. (1934). In 1918 at Oaks Lake about 20 miles from Windom, Minnesota, a shallow lake of some 400 acres with a depth of about 5 feet, death of domestic animals occurred involving some 50 chickens, 17 hogs, and 1 sheep. The algae present in the water that were predominate at the time of deaths were: *Coleosphaerium Kuetzingianum* Näg., and *Anabaena flos-aquae* (Lyngb.) Bréb. As in other similar instances, wind blowing over the lake had concentrated the algae along the shore. The second investigation of domestic animals killed by toxic algae made by the Division of Veterinary Medicine of the University of Minnesota occurred in 1930 at Lake Anne near the village of Harvard Lake, Minnesota. Here a wind had concentrated algae along the shore. Nine cattle were killed from drinking water in which *Microcystis flos-aquae* (Wittr.) Kirch. and *M. aeruginosa* Kütz. were predominant. Fitch et al (1934) reported that during the investigations carried out in connection with this killing that large quantities of algae collected shortly after cattle fatalities when fed to rabbits, guinea pigs, and cattle produced no ill effects. Macerated tissues from animals which had died of toxic algae poisoning when injected into guinea pigs and rabbits caused neither sickness nor death. In 1933 the veterinarian division was called again to investigate deaths from water blooms, this time consisting of *Microcystis flos-aquae* at Hall Lake, south of Fairmont, Minnesota. Animals which had died after partaking of this water were sheep and chickens. On September 28, 1933 another domestic animal killing at Lake Lac qui Parle at Milan, Minnesota was investigated where 45 turkeys, 4 ducks, and 2 geese were the victims of toxic algae including *Microcystis flos-aquae*, *Anabaena flos-aquae*, and

Aphanizomenon flos-aquae. A second killing took place in this lake on October 4, 1933, involving the same species of algae. Animals reported killed at this time were cows, pigs, horses, and poultry. In 1933 three cattle were reported to have been killed from a toxic algal bloom consisting of *Microcystis* and *Anabaena* in the vicinity of Grand Rapids, Minnesota.

Howard and Berry (1933) reported the death of about 20 cattle at Fraser Lake, Hastings County, Ontario, Canada, attributable to *Anabaena*.

Steyn (1945) reports that the first cases of poisoning with algae in South Africa were observed in stock, rabbits, and water birds in the Amersfoort district of South Africa in 1927. He further states that in the northeastern Orange Free State and southeastern Transvaal, many thousands of stock, principally sheep and cattle, have died from being poisoned by toxic algae in the past 25 to 30 years. In these deaths, species of *Microcystis* are indicated as the offenders. Steyn (1945) has noted that if large quantities of algae-bearing water are consumed, animals die from acute general paralysis or with strychnine-like convulsions. If only small quantities of contaminated water are consumed by cattle, however, he implies that only illness occurs, accompanied by the following symptoms: 1) certain degree of constipation; hard feces, covered with blood; 2) drop in milk yield; 3) general weakness; 4) signs of skin burning by sun (photosensitization); 5) more protracted cases show jaundice as a result of liver injury.

Steyn (1945) suggests that the following treatment should be applied to animals poisoned by algae: enemata; purgatives; heart stimulants; provision of shade; liniments for skin lesions.

The alga (a new species) concerned with the poisoning of cattle in South Africa was described by Stephens (1948) as *Microcystis toxica*, widespread in southeast Transvaal and in northeast Orange Free State. She states that the alga produces one of the most powerful known liver poisons, killing thousands of animals between 1940 and 1945. The plants are less poisonous when dried, however, than in the living condition.

Prescott (1948) reported death of cattle in Bermuda after they had taken water from pools and troughs in which *Aphanizomenon flos-aquae* had formed a dense bloom.

Louw (1950) reported several cases of poisoning of livestock in South Africa where a blue-green alga, *Microcystis toxica* Stephens, growing abundantly in the water was thought to be the responsible organism.

In concluding this discussion of field observations on the effects of toxic algae, it may be said that commonly the symptomatology of poisoning indicated prostration and convulsions followed by death. Francis (1878) vividly described the symptoms of cattle poisoned by algae: ". . . symptoms—stupor and unconsciousness, falling and remaining quiet, as if asleep, unless touched, when convulsions come on, with head and neck drawn back by rigid spasm, which subsides before death."

Laboratory Studies on the Effects of Toxic Algae on Mammals and Birds

Fitch et al (1934) observed that when samples of toxic algae were fed in the fresh state to guinea pigs, rabbits, and chickens, death occurred in a very short time. When toxic algae were stored for a short time at ice box temperatures, or dried, it was further observed that it was almost impossible to produce

death by feeding, but with a similar dose, animals could be killed quickly by inoculating intraperitoneally. Fitch et al (1934) do not list the specific alga or algae used.

The syndrome of symptoms described by Fitch et al (1934) exhibited by guinea pigs from the feeding or from the inoculation intraperitoneally of a fatal dose of toxic algae is: 1) restlessness; 2) urination; 3) defecation; 4) deep breathing; 5) weakness in the hind quarters; 6) sneezing; 7) coughing; 8) salivation; 9) lachrymation; 10) clonic spasms and death.

In studying experimental deaths of guinea pigs, it was noted that they occasionally show symptoms of intoxication and then recover. The syndrome of symptoms in pigeons observed by Fitch et al (1934) is: 1) immediate restlessness; 2) immediate blinking of eyes; 3) immediate repeated swallowing, at times accompanied by regurgitation; 4) in from 9 to 10 minutes loss of balance; falling on breast; head drawn back; 5) clonic spasms and death.

Wheeler et al (1942) working with animals and fresh *Microcystis aeruginosa* filtered from sample water and resuspended in just enough water to flow through a sixteen gauge hypodermic needle made the following observations: 1) mice invariably killed when either a subcutaneous or intraperitoneal dose was more than 0.25 ml., death occurring rarely in less than sixteen hours or in more than thirty-six; 2) a guinea pig given a 2 ml. inttraperitoneal dose died in twenty-four hours; 3) a rabbit given a 5 ml. intraperitoneal dose showed no ill effects; 4) guinea pigs given 4 ml. oral doses showed no ill effects; 5) mice given algae orally by a blunt needle or by adding algae to drinking water nearly always survived; 6) injections of pond water after the alga was filtered off did not affect animals.

The above investigators found that *Microcystis aeruginosa* that had been preserved by quick freezing was far more lethal to mice when thawed out than was fresh material. A minimum lethal dose for mice of 0.25 ml. portions of the thawed out alga was established by injection. Also, the toxic substance went into solution, for filtrates of diluted frozen algae were as toxic to mice on injection as was the unfiltered material. Mice that were given a lethal dose of preserved material subcutaneously behaved normally for half an hour, then had periods of apathy alternating with an exaggerated response to stimuli and restlessness, followed by rapid and superficial respiration just before death occurred. Between periods of apathy and restlessness the eyes changed to a lighter pink and the ears and tails turned chalky-white.

Olson (1949) writes that cultures of *Microcystis aeruginosa* were demonstrated to be toxic to mice. Later (1952) he points out that the toxicity of unpurified algal material from field collections varies a great deal. For example, 0.02 ml injected into mice intraperitoneally may kill a 20 gram individual in an hour, whereas of another sample it may require 2.0 to 3.0 ml to produce death in 18 hours. Certain samples of toxic algae (0.1 ml fatal dose) taken from a lake one day may kill mice in 20 minutes, whereas nine days later, samples from the same lake with a greatly increased dosage (2.0 ml) may require five hours to produce death. Shifts in ratios of predominant toxic algae in lake water may partially explain the above phenomenon; however, other factors also may be involved.

Mason and Wheeler (1942) investigated the effects of partially purified extracts of the alga, *Microcystis aeruginosa* on mice and cats. They observed that following subcutaneous or intraperitoneal injections of a lethal

dose, (equivalent to 3 milligrams of algal dry weight for mice and approximately 1 gram for a 1500 gram cat) there is a twenty minute latent period for mice and from two to three hours for cats. Latency is followed by pallor, reduced blood pressure, tachycardia, reduced body temperature, hyperglycemia, a marked reduction in hemoglobin, red count, and total serum protein. Death results from respiratory failure. Autopsies were made and results were recorded. Experimental work was done to show certain effects of the toxic material on perfused frog's heart and on various organs of guinea pigs and mice.

Ashworth and Mason (1946) studied pathological changes produced in organs of rats by the toxic agent in *Microcystis aeruginosa* following the administration of maximal sublethal dose of algal extract. They state that the toxic substance from the alga upon administration to rats leads to generalized cellular damage with particularly severe injury to parenchymal cells of the liver. Early death caused by acute poisoning can probably be attributed to shock and circulatory collapse, while delayed death is probably consequent to hepatic insufficiency, renal failure, and generalized cellular damage by the toxic agent. They finally note that types of structural changes in the liver produced by the toxic substance are similar in many respects to those produced by several known hepatotoxic substances.

Wheeler et al. (1942) using mice as test animals attempted to determine the extent to which toxic material from *Microcystis aeruginosa* would survive in aqueous solutions subjected to various types of water treatment. They state that the limited amount of material available made it impossible to make tests comparable to regular procedures in use in water treatment plants, however such procedures were followed as nearly as possible with available laboratory equipment. In their summary these workers write that the toxic substance survives the laboratory equivalent of water treatment, using alum coagulation, chlorination, and filtration. Olson (1952) found that activated carbon in amounts corresponding to those used in water treatment was not effective in removing toxic materials from algae in water, although large amounts made the effluent non-toxic to mice in 1.0 ml doses. This writer further states that algal toxins survive even after massive treatment with Norite A. He does not specifically state which algae or alga he worked with.

Toxicity of Algae to Fish

Prescott (1948) has presented evidence that suggests the possibility of fish deaths being caused by the toxic action of decomposing *Aphanizomenon flos-aquae*. Experimental data gathered by him indicate that the fish used in his experiments were not killed by a lack of dissolved oxygen, as might be the case when environmental conditions destroy algal blooms. Under natural conditions the algae would be decomposed by bacterial action which would reduce dissolved oxygen to critical levels, resulting in fish deaths. In one instance, Prescott (1948) found that fish deaths resulted under experimental conditions when dissolved oxygen varied from 4 to 6 ppm during the course of the experiment. To make this test, 20 gallons of decomposing *Aphanizomenon flos-aquae* were added to aquaria, killing all fish but one crappie within six hours. The fish killed were crappie, *Pomoxis nigro-maculatus* L. S., imported gizzard shad, *Dorosoma cepedianum* L. S., golden shiner, *Notemigonus crysoleucas* Raf., orange-spotted sunfish, *Lepomis humilis* Gir., fathead minnow, *Pimephales p. promelas* Raf., bluegill, *Lepomis macrochirus* Raf., and

buffalo, *Megastomatobus cyprinella* Val. Another experiment with the same alga in which the D. O. was satisfactory for fish involved the addition of about 5 gallons of decaying algae to an aquarium, producing death in the following fish species within five hours: sheephead, *Aplodinotus grunniens* (Raf.); minnow, *Hyborhynchus notatus* Raf., perch, *Perca flavescens* (Mitchell); bullhead, *Ameiurus m. melas* Raf.; pumpkinseed, *Lepomis gibbosus* L.; and carp, *Cyprinus carpio* L. In the field Prescott (1948) prepared small ponds on the shores of Storm Lake, Iowa, into which he introduced lake water heavily loaded with *Aphanizomenon flos-aquae*. The algae were allowed to decay in the shallow water. Dissolved oxygen was at 7.5 ppm initially and at no time during his observations dropped low enough to cause fish suffocation. The fish that were introduced into these ponds died at the end of two hours. Species used in this experiment were the crappie: *Pomoxis nigro-maculatus*, carp., *Cyprinus carpio*, and bullhead, *Ameiurus m. melas*. In attempting to explain the various fish deaths Prescott (1948) presented the following data: "Decomposed blue-green algae were analyzed chemically. It was found that a poisonous protein decomposition product, hydroxylamine, was present in quantities to kill fish. Also sufficient amounts of hydrogen sulphide to kill fish were found to be given off in decomposing algae. Quantitative tests showed 8.5 ppm of H_2S in the sample analyzed. The writer was informed by the analyst that this figure was far below the actual concentration because much of the gas was lost during distillation. How sensitive fish are to H_2S, the writer is unable to state, but, suffice to say, this gas is poisonous and could have been liberated in quantities sufficiently great to kill fish exposed to great masses of decaying algae in shallow water."

Mackenthum et al. (1948) conducted experiments to show the toxic effects of water that had contained decomposing *Aphanizomenon flos-aquae* on certain fish. Yellow perch, crappies, and a common sucker were killed in aquaria containing aerated water taken from the Yahara River, Wisconsin, where fish were dying in the stream from an apparent depletion of the oxygen supply brought about by the decaying alga. During the aquarium experiments the dissolved oxygen was maintained at a suitable level for fish. Based on their experimental work and field data these investigators wrote in relation to fish deaths and the above alga, stating: "It is concluded from the temperature and chemical data and from the results of experiments that the primary cause of the fish mortality was the depletion of the oxygen supply [in the Yahara River] brought about by decomposition of a huge mass of *Aphanizomenon flos-aquae*. Secondarily, toxic elements released by the decomposing algae probably increased the mortality."

Prescott (1939) in writing on some limnological relationships of phytoplankton to aquatic biology made the following comments on blue-green algae as they may relate directly to fish deaths, ". . . it is apparently possible for algae to bring about the death of fish through the liberation of substances toxic to them during the decay process. When highly proteinacious blue-green algae undergo decay, sufficient quantities of hydroxylamine and other derivatives are produced to poison any fish caught in the shallow water of a bay by masses of decaying algae."

CHEMISTRY OF TOXIC ALGAE

Fitch et al. (1934) first described the chemical characteristics of certain

blue-green algae suspected of causing domestic animal deaths. Tests for cyanide poisoning metals proved negative; extraction methods for alkaloids were negative. In using guinea pigs as indicators of the presence and potency of toxic algal material as well as of chemical analyses, it was concluded that the poison was: 1) non-volatile; 2) heat stable in dried state, 102-104°C for 100 hours; 3) unstable to heat when wet 100°C for any appreciable period, unless cells are freshly taken from water; 4) able to retain potency when air dried; 5) around algal cells in lake water; 5) not an electrolyte, or is not electrically charged for no migration is observed under an electrical potential; 7) has small molecular weight; will pass through ultra filters such as colloidian bags or cellophane tubes; 8) absent after putrefaction of the algae; 9) sometimes resistant to acidic or alkaline conditions somewhat removed from acidity of water in which it is produced; 10) nonreactive chemically either as an alkaloid or as a toxalbumin; 11) not botulinus toxin; not neutralized by polyvalent botulinus anti-toxin; 12) an organic compound according to tests.

In working with *Microcystis aeruginosa,* Wheeler et al. (1942) in an attempt to find a clue to the chemical nature of the poison produced by this alga, made the following observations: 1) dialysate concentrated *in-vacuo* and residue dried in vacuum oven, an amorphous, brown, somewhat oily substance of high toxicity is obtained; no crystals observed; m.l.d. mouse is 0.004 mg; 2) water and alcohol extracts toxic; ether, benzene, chloroform, non-toxic; 3) toxic substance stable only to heat (boiling or autoclaving) in neutral solution; destroyed by heat in 1 per cent alkaline or acid solution; 4) no toxicity loss (autoclaved) solution in a month; unsterile solutions with gradual potency loss; 5) toxic substance adsorbed readily by activated carbon; filtrate from carbon non-toxic, but carbon residue toxic for mice, action being delayed some 10 hours; 6) following tests all negative: for carbohydrates, Benedict; for amines, quinone; for proteins, millon; for cyanides, sodium picrate. These tests run on dilute solutions because of limited algal supply, thus not conclusive; 7) on solubility basis determined from extract toxicity, possibility that toxicity was caused by one or the other of the following compounds can be ruled out; cyanogenetic glucosides, amygdonitrile glucoside, or amygdalin; 8) not an organic ester such as acetycholine.

Wheeler et al. (1942) state: "There is some evidence that the same algae may be toxic at one time and not at another—as though the toxic phase might be a stage of development or of decomposition."

Steyn (1945) writes in relation to the toxic principles of *Microcystis:* "This alga appears to contain two active principles, namely, a most potent liver poison and photosensitizing agent (phycocyanin)."

Prescott (1948) recorded the following data from a chemical analysis of decomposing blue-green algae: Poisonous protein decomposition product, hydroxylamine, was present (in sufficient quantities to kill fish).

Louw (1950) found that the toxic substance from *Microcystis toxica* was a liver poison, was alkaloid in nature, and was successfully isolated as the picrate.

Olson (1952) comments that the crude toxic principle from the blue-green algae possesses the following characteristics: 1) can exist outside cells in water around algae; 2) passes through cellophane and animal membranes by dialysis; 3) is non-volatile; 4) is relatively heat stable, a —no appreciable loss of toxic-

ity in dry preparations at 100°C for 100 hours, b—no appreciable loss in toxicity in wet preparations autoclaved at 15 lbs. for 90 minutes; 5) soluble in water, alcohol (95% or less), acetone; 6) non-soluble in benzene, ether, chloroform; 7) resistant to extreme changes in pH; 8) specific tests for following compounds negative; cyanide, nitrate, nitrite, pilocarpine, saponins, Legal's test for cardiac glycosides; extraction procedures for pilocarpine and strychnous alkaloids negative.

SUMMARY

Outbreaks of human gastroenteritis have not been positively traced to algae.

Algae that have been responsible for mammalian, avarian, and fish deaths through some toxic action are all to be found in the blue-green algal group, the Cynapophyta. The Cyanophyta species that have been associated with animal deaths belong in the genera: *Microcystis, Aphanizomenon, Anabaena, Nodularia, Coelosphaerium,* and *Gloeotrichia.*

Often when deaths of animals occur, a wind has been reported blowing, thus tending to concentrate algae in lee-shore areas.

Cattle that drink only small quantities of water containing *Microcystis* may not die but do show a series of illness symptoms, one of which is a drop in milk yield.

Symptomatic treatment has been recommended by Steyn (1945) for cattle poisoned by algae.

Various writers have made reference to several toxic substances associated with blue-green algae.

Substances that are toxic enough to cause illness or death in animals are not present in all blue-green algae.

Water in which certain blue-green algae have bloomed may produce death in mammals and fish when the algal cells themselves are excluded.

The toxic material from certain algae may survive the laboratory equivalent of water treatment, using alum coagulation, filtration and chlorination. It may survive activated carbon treatment in amounts corresponding to that used in water treatment plants, and after massive treatment with Norite A.

REFERENCES

ARTHUR, J. C. 1883—Some algae of Minnesota supposed to be poisonous. Bull. Minn. Acad. Sci. 2(Appendix):1-12.

———1886—Some algae of Minnesota supposed to be poisonous. 4th Biennial Rep., Board of Regents, Univ. Minn., Suppl. 1, Dept. of Agri.:97-103.

———1886a—Second report on some algae of Minnesota supposed to be poisonous. Ibid. Suppl. 1:109-112.

ASHWORTH, C. T. AND M. F. MASON 1946—Observations on the pathological changes produced by a toxic substance present in blue-green algae (Microcystis aeruginosa). Amer. Journ. Pathology 22:369-384.

BRANDENBURG, T. O. AND F. M. SHIGLEY 1947—"Water bloom" as a cause of poisoning in livestock in North Dakota. Jour. Amer. Vet. Med. Assoc. 110:384.

COTTON, H. L. 1914—Algae poisoning. Amer. Jour. Vet. Med. 9:903-904.

DAILY, W. A. 1942—The Chroococcaceae of Ohio, Kentucky, and Indiana. Amer. Midl. Nat. 27(3):636-661.

DEEM, A. W. AND F. THORP 1939—Toxic algae in Colorado. Jour. Amer. Vet. Med. Assoc. 95:542-544.

DROUET, F. AND W. A. DAILY 1939—The planktonic fresh-water species of *Microcystis.* Field Mus. Nat. Hist. Bot. Ser. 20(3):67-83.

FITCH, C. P., L. M. BISHOP ET AL. 1934—"Water bloom" as a cause of poisoning in domestic animals. Cornell Veterinarian 24(1):30-39.

FRANCIS, G. 1878—Poisonous Australian lake. Nature 18:11.
GILLAM, W. G. 1925—The effect on livestock of water contaminated with fresh-water algae. Jour. Amer. Vet. Med. Assoc. 20:780-784.
HOWARD, N. J. AND A. E. BERRY 1933—Algae nuisances in surface waters. Canad. Pub. Health Jour. 24(7):377-384.
LOUW, P. G. J. 1950—The active constituent of the poisonous algae, *Microcystis toxica* Stephens. So. African Indust. Chem. 4:62-66.
MACKENTHUM, K. M., E. F. HERMAN AND A. F. BARTSCH 1948—A heavy mortality of fishes resulting from the decomposition of algae in the Yahara River, Wisconsin. Trans. Amer. Fisheries Soc. 75(1945):175-180.
MASON, M. F. AND R. E. WHEELER 1942—Observations upon the toxicity of blue-green algae. Fed. Proc. Amer. Soc. Exp. Biol. 1:124.
NELSON, N. P. B. 1903-04—Observations upon some algae which cause "water bloom." Minn. Bot. Stud. 3(1903-1904), Bot. Ser. VI:51-56.
OLSON, T. A. 1949—History of toxic plankton and associated phenomena. Sew. Works Eng. 20(2):71.
———1952—Toxic plankton. Water and Sewage Works 99:75-77.
PORTER, E. M. 1886—Investigation of supposed poisonous vegetation in the waters of some of the lakes of Minnesota. 4th Biennial Rep., Board of Regents, Univ. Minn., Suppl. 1, Dept. of Agric.:95-96.
PRESCOTT, G. W. 1933—Some effects of the blue-green algae, *Aphanizomenon flos-aquae* on lake fish. The Collecting Net 8(4):77-80.
———1939—Some relationships of phytoplankton to limnology and aquatic biology. *In* Problems of Lake Biology, Amer. Assoc. Adv. Sci. Pub. No. 10:65-78.
———1948—Objectionable algae with reference to the killing of fish and other animals. Hydrobiologia 1(1):1-13.
———1951—Algae of the western Great Lakes area. 946 pp. Cranbrook Press.
QUIN, A. H. 1943—Sheep poisoning by algae. Jour. Amer. Vet. Med. Assoc. 102:299.
SCOTT, R. M. 1952—Algal toxins. Public Works, March:54-55, 65-66.
SMIT, J. D. 1950—Experimental cases of algae poisoning in small animals. So. African Indust. Chem. 4:66.
SMITH, G. M. 1950—The fresh-water algae of the United States. 719 pp. McGraw-Hill Book Co.
STALKER, M. 1886—Stalker on the Waterville cattle disease. 4th Biennial Rep., Board of Regents, Univ. Minn., Suppl. 1, Dept. of Agric.:105-108.
STEPHENS, EDITH L. 1948—*Microcystic toxica* sp. nov. a poisonous alga from the Transvaal and Orange Free State. Hydrobiologia 1(1):14.
STEWART, A. G., D. A. BARNUM AND J. A. HENDERSON 1950—Algal poisoning in Ontario, Canada. Canad. Jour. Comp. Med. 14:197-202.
STEYN, D. G. 1943—Poisoning of animals by algae in dams and pans. Farming in South Africa 18(208).
———1944—Poisonous and non-poisonous algae (waterbloom, scum) in dams and pans. Ibid. 19(220).
———1945—Poisoning of animals and human beings by algae. So. African Jour. Sci. 41:243-244.
———1945a—Poisoning of animals by algae (scum or waterbloom) in dams and pans. Union of So. Africa, Dept. of Agric. and Forestry. 9 pp. Pub. at Pretoria, South Africa.
TISDALE, E. S. 1931—Epidemic of intestinal disorders in Charleston, West Virginia, occurring simultaneously with unprecedented water supply conditions. Amer. Jour. Public Health 21:198-200.
———1931a—The 1930-31 drought and its effect upon water supply. Ibid. 21:1203-1215.
VELDEE, M. V. 1931—Epidemiological study of suspected water-borne gastroenteritis. Ibid. 21:1227-1235.
WHEELER, R. E., J. B. LACKEY AND S. SCHOTT 1942—A contribution on the toxicity of algae. Pub. Health Rep. 57(45):1695-1701.
WOODCOCK, E. F. 1927—Plants of Michigan poisonous to livestock. Jour. Amer. Vet. Med. Assoc. n.s., 25:475.
WURTZ, A. 1949—Proprietes particuliérés d'une fleur d'eau de Cyanophycee: Microcystis aeruginosa Kütz. Bull. de La Soc. Botanique de France 96:49-50.

Cytotaxonomical Studies on the Northern Bedstraw

Askell and Doris Löve
University of Manitoba, Winnipeg, Canada

Although the genus *Galium* is one of the larger genera of the higher plants, including more than 250 species (cf. Schumann, 1891; Willis, 1949) distributed throughout both hemispheres, it does not as yet seem to have found its present day monographer. Several of its critical taxa have been studied separately by different botanists. However, in recent manuals, the same author may treat some of the species of the genus in the sense of the splitter, while others are considered from the point of view of the lumper (cf. Fernald, 1950; Hultén, 1949). These differences in opinion about the various taxa of the genus are certainly due only to the fact that most of the subgroups already described are found to differ in but slight technical characters, and, as most of the species are small, their minute morphological differences are not always readily observed.

According to Schumann (1891), the genus should be naturally grouped into at least fourteen different sections, some of which are divided into subgroups. Each of these groups is morphologically very distinct, while only a few minor characters are found to be sufficient to distinguish the species of each section or subsection. Nevertheless, the rather morphologically related species are found to have a distinct area of distribution, hybrids between them are rare or even absent, and, in cases where they have been studied from the cytogenetical points of view, species even morphologically very closely related or types previously known as varieties by taxonomists, have been found to have a strong barrier of hybrid sterility or incompatibility based on differences in the number or structure of their chromosomes (cf. Homeyer, 1935; Fagerlind, 1937; Ehrendorfer, 1949, 1951; Poucques, 1949).

The perennial section *Platygalium* DC. includes about ten species differing in a few technical characters only. Some of these certainly are valid species and include but one ecospecies in the sense of the modern biosystematists (cf. Turesson, 1922, 1925, 1930, 1938; Clausen, Keck, and Hiesey, 1940, 1945), while the morphological as well as the geographical variability of some of the others indicate that they are collective species which might be given a more correct taxonomical status on the basis of cytotaxonomical or genecological analysis. One of these species is the so-called Northern Bedstraw, or the collective species *Galium boreale* of all recent manuals covering different parts of the northern hemisphere.

The species *Galium boreale* was originally described by Linnaeus (1753) from northern Europe ("Habitat in Europae borealis pratis"). In that outstanding "Atlas of the Distribution of Vascular Plants in N.W. Europe," Hultén (1950) classifies it as one of the boreal-circumpolar plants lacking large gaps in their area. Notwithstanding this schematical classification, the species shows a very clear gap in its distribution from southern Quebec to Iceland (cf. Böcher, 1938; Fernald, 1950; Ostenfeld, 1926; Raymond, 1950; Scoggan, 1950), and the ecological requirements of the North American and European populations seem to be somewhat different. In Scandinavia (cf. Hultén, 1950; Lid, 1944; Nordhagen, 1940; Dahl, 1934; Kotilainen, 1951), the British Isles (cf. Druce, 1930; Webb, 1943), as well as in the Faeroes (cf. Rasmussen, 1936) and Iceland (cf. Löve, 1945; Stefánsson & Steindórsson, 1948) it is met with in alpine as well as rather maritime and inland subarctic

conditions, while in North America it seems to avoid the arctic-subarctic and maritime regions (cf. Hultén, 1949; Polunin, 1940; Porsild, 1951).

Morphologically, *Galium boreale* is so highly variable in the breadth and degree of ciliation of leaves as well as in its stature and degree of branching so as to prevent most attempts at organization of its vegetative variations on basis of these characters. In the hairiness of the fruits, which offers a good specific difference in other sections of *Galium* when accompanying other characters, the species *G. boreale* s. lat. is extremely variable in that the carpels range from strictly glabrous to densely villous-hirsute. Nevertheless, the hairiness of the fruits seems to be one of the few characters used in order to classify the variations of the species. The typical plant, *G. boreale* L. var. *boreale* (var. *typicum* Beck, 1893; var. *genuinum* Grenier & Gordon, 1847-52; var. *scabrum* DeCandolle, 1830) has fruits villous-hirsute with long hairs, the var. *hyssopifolium* (Hoffm.) DC. (cf. DeCandolle, 1830) has glabrous or glabrate fruits, while var. *intermedium* DC. (cf. DeCandolle, 1830) has fruits covered with short appressed or incurving hairs. While these varieties or forms seem to be rather intermixed in Europe without any marked differences in distribution or ecological requirements, they have been observed to present some of the main characteristics of geographical races in North America. Thus, the main type with villous-hirsute fruits is common in the northwestern parts of the continent, from Manitoba to New Mexico and westwards and northwards to the Cordilleran region and Alaska (cf. Hultén, 1949). The type with appressed or incurving hairs is known from the Cordilleran region eastwards to New England, although it is much less frequent in the eastern than in the western parts of the continent. The type with glabrous or glabrate fruits, however, is mainly eastern and seems to be the only type occurring in Gaspé (cf. Scoggan, 1950), Nova Scotia (cf. Roland, 1944), and northern New England (cf. Fernald, 1950). It ranges westwards through southern Quebec to Manitoba and North Dakota, and then locally to New Jersey, Ohio, Missouri, Colorado, as well as to some localities on the Pacific coast in Washington, Oregon, and Vancouver Island (cf. Fernald, 1928).

Although contemporary writers of manuals seem to regard it as most convenient to treat the Northern Bedstraw only as a collective species with a few more or less critical varieties, this has not always been so. More than a century ago, Roemer & Schultes (1818) described the North American representatives as a separate species under the name *Galium septentrionale* R. & S. The following year Torrey (1819) described the material from New York as *G. strictum* Torr. The new species of Roemer & Schultes was accepted by Bigelow (1824), as well as by DeCandolle (1830). The American type was, however, regarded as being identical with the European species by Pursh (1814), Hooker (1840), and Darlington (1837). Even Torrey (1824, 1843) himself later accepted this view, as have all the subsequent authors of manuals on North American plants.

Problems like the one of correct taxonomical subdivision of the collective species *G. boreale* are ideal ones for the biosystematist. Cytotaxonomical studies of types from different parts of the distribution area of each taxon might reveal if there are any sterility barriers between morphologically distinguishable types. Closer genecological studies of material from ecologically different places inside each of the perhaps genetically isolated types may then reveal what is the real taxonomical position of morphologically more or less

distinct intraspecific variations, as well as indicate the evolutionary history of the complex as a whole. The present paper gives the results of an enquiry into the first-named category only.

MATERIAL AND METHODS

The living material studied cytologically and morphologically originated in part from different localities in Iceland and southern Sweden, and partly from localities in the province of Manitoba, from Arborg in the north, Fort Garry in the south, Delta in the west, and Vivian in the east. The morphological variations were amplified with studies on herbarium material from different parts of the distribution area of the collective species, mainly in the herbarium of the University of Manitoba, the private herbarium of the writers, as well as from the Botanical museum of the University of Copenhagen, lent by Dr. O. Hagerup.

For the determination of chromosome numbers the writers used exclusively mitotic material in permanent section slides. This made the task somewhat more tedious though at the same time the determinations were bound to be much more exact and certain than if smear preparations of meiotic or mitotic divisions had been used. The cytological studies were based on root-tip material fixed in nature in the following modification of the Navashin-Karpechenko's fixative: I. 1 g chromic acid, 10 cm^3 acetic acid, 85 cm^3 water, distilled. II. 30 cm^3 formaldehyde, 40%, 10 cm^3 alcohol, 95%, 55 cm^3 water, distilled. Solutions I and II are kept separate and mixed in equal proportions just before fixing of the root-tips. The material can then be stored in the solution for years, and it should not be disturbed during the first 24 hours. After this minimum delay the tips are washed in water and the fixative in the vials replaced with 70% alcohol.

For dehydrating the fixed material, the following schedule was used: After at least 1 hour in 70% alcohol, the following mixtures of alcohol are changed by steps: 95% alcohol for 1 hour; alcohol abs. for 1 hour; n-butyl alcohol: alcohol abs. in the proportion of 1:2 for ½ an hour; n-butyl alcohol: alcohol abs. in the proportion 2:1 for ½ an hour, and then n-butyl alcohol, to which paraffin (melting point about 55°C) is added. The dish with this mixture is placed in a thermostat oven with about 60°C over night or until the smell of butyl is no longer perceptible. Then the root-tips are embedded in the paraffin on watch-glasses in the usual way.

The root-tips were generally sectioned about 12 micra thick and stained according to the Newton crystal violet method (cf. Newton & Darlington, 1929; Darlington & La Cour, 1942).

The measurements of seeds and other organs were taken under the microscope on unstained material.

The specimens studied cytologically were preserved in the herbarium of the University of Manitoba, in Winnipeg.

MORPHOLOGY

At first sight, the Canadian material of the Northern Bedstraw was found to differ somewhat from the Icelandic and Scandinavian materials in its general appearance, scarcely describable in technical terms. As no distinct character for separating them morphologically was known to the authors, they searched for some technical and measurable characteristics.

The average height of the Manitoban plants is greater than that of the European material hitherto studied in the field by the authors. As the latter material is, however, only from northern Europe, this difference might be due only to differences in climate and the length of daylight. The height of some French herbarium plants seen by the authors seems to support this suggestion.

The stems of the individuals from America as well as from Europe are strictly four-angled and the internodes are quite glabrous. The nodes of the American specimens are, however, clearly puberulent, with a thick felt of very short and stiff hairs. This character seems to be absent from the European and western Asiatic material hitherto investigated, although some individuals from scattered localities in Europe were found to have some soft and scattered hairs on some of the upper nodes.

The types and length of the leaves are highly variable, as is also their green color. Although linear leaves seem to be more frequent in Europe than in North America, almost lanceolate leaves are sometimes met with in populations from both the continents. The leaves are always trinerviate. The variation in their pilosity is so wide on both sides of the Atlantic as to make it a character of no taxonomical value. The bracts might, perhaps, be of some value in this connection as they are found to be circular or ovate and obtuse in the European material studied, while those in the American specimens are oblong and acute.

The panicle shows a wide variation in type and size in specimens from different localities in both regions. Its size and type are, perhaps, determined more by climatic than by genetic factors. Although this cannot be confirmed in measurements, the American specimens, as a whole, however, are found to have a more divaricate and open panicle than do the European individuals.

On both continents the flowers vary somewhat in size. According to Lid (1944) they are about 3 mm wide in Norway, while Stevens (1950) gives only 2 mm for the width of the flowers in North Dakota. Measurements made by the present writers on some herbarium material from Europe and North America (cf. table 1) indicate, however, that although the width of the corolla is variable in both regions, the European specimens have distinctly ($P < 0.001$) less wide flowers than the American ones. The measurements were made on freshly though widely opened flowers from the lowest part of the panicle of individuals collected in late July. And only completely whole and opened corollas were used in the measurements.

The color of the flower is white, but there is a distinct difference in the shade between European and American material. The petals of the American plants are bright white (cf. Fernald, 1950), while the European specimens always have somewhat creamy petals. This difference, which is certainly based upon some chemical dissimilarities, is sometimes even observable in well-preserved herbarium material.

As mentioned above, the hairiness of the fruit of the American plants shows the same variability as does the hairiness of the fruit in Eurasia. Closer studies of this character were not made by the present authors, as such an investigation needs more herbarium material as well as genecological experiments on a vast scale.

The size of the fully mature fruits was measured on several specimens from North America and Europe (cf. table 2). Although the average difference in length of the fruits might seem to be inconsiderable, it is statistically

TABLE 1.—Width of corolla (Measurements in 0.1 mm)

	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	n	M ± m
Iceland	..	..	1	1	4	5	6	16	8	3	3	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..		
Scotland	1	1	3	7	6	8	11	19	14	8	5	2	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..		
Denmark	..	..	1	3	7	11	15	17	13	9	5	2	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..		
Sweden	1	1	..	1	3	4	9	14	8	5	3	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..		
Germany	..	1	1	4	9	12	19	22	17	14	9	5	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..		
Switzerland	1	..	1	2	4	7	9	12	8	8	4	3	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..		
Caucausus	..	..	..	2	5	9	11	15	9	8	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..		
Jeniseisk	..	1	1	2	4	9	14	18	12	11	4	1	2	1	..	..	..	..	..	..	..	..	..	..	..	..	..		
Total	3	4	8	22	42	64	94	133	89	66	36	16	6	4	..	..	..	..	..	..	..	..	..	..	..	..	.	687	36.9 ± 0.01
New York	..	..	..	..	..	..	..	..	..	..	1	..	2	3	7	8	12	17	13	8	8	4	2	1	1	..	..		
Wyoming	..	..	..	..	..	..	..	..	1	1	1	3	4	7	12	15	19	23	19	17	9	7	5	2	1	2	1		
Manitoba	..	..	..	..	..	..	..	..	1	3	5	9	13	14	20	33	37	43	38	31	18	17	12	8	5	4	1		
Alberta	..	..	..	..	..	..	..	..	..	1	1	2	4	4	7	9	14	19	12	10	7	3	4	2	1	..	1		
Yukon	..	..	..	..	..	..	..	..	..	1	1	1	2	3	8	11	12	17	9	10	7	5	2	1	2	1	..		
Alaska	..	..	..	..	..	..	..	..	1	..	1	2	3	4	8	6	12	15	10	7	4	3	1	1	..	1	..		
Yunnan	..	..	..	..	..	..	..	..	..	1	1	3	7	7	9	15	19	22	17	13	9	8	6	4	1	2	1		
Kashmir	..	..	..	..	..	..	..	..	..	1	2	3	4	8	9	14	17	21	16	15	9	5	2	1	1	1	..		
Turkestan	..	..	..	..	..	..	..	..	..	..	1	1	3	6	6	7	13	16	11	9	7	5	2	2	1	..	1		
Total	..	..	..	..	..	..	..	..	3	8	14	24	42	56	86	118	155	193	145	120	78	57	36	22	13	11	5	1186	46.9 ± 0.01

TABLE 2.—Length of mature fruits (Measurements in 0.01 mm)

	150	155	160	165	170	175	180	185	190	195	200	205	210	215	220	225	230	235	n	M ± m
Iceland	6	17	22	33	37	43	35	34	25	15	5	..	..	..	..	..	..	..		
Scotland	1	2	7	10	17	22	15	12	6	3	1	..	..	..	..	..	..	..		
Denmark	2	5	11	19	27	34	25	20	9	5	1	..	..	..	..	..	..	..		
Sweden	4	17	29	36	43	59	47	34	28	14	7	..	..	..	..	..	..	..		
Germany	7	14	29	37	51	67	49	41	33	17	5	..	..	..	..	..	..	..		
Switzerland	1	3	9	17	19	23	18	17	7	2	1	..	..	..	..	..	..	..		
Caucausus	..	1	3	9	17	29	21	7	2	1	..	..	..	..	..	..	..	..		
Jeniseisk	1	5	12	19	27	39	25	21	10	6	1	..	..	..	..	..	..	..		
Total	22	64	122	180	238	316	235	186	120	63	21	..	..	..	..	..	..	..	1567	171 ± 0.1
New York	..	..	..	..	..	..	6	20	38	58	69	89	72	56	39	19	8	1		
Wyoming	..	..	..	..	..	1	4	15	22	33	37	42	35	34	23	13	3	1		
Manitoba	..	..	..	..	..	1	9	22	41	73	92	112	95	69	40	19	7	2		
Alberta	..	..	..	..	..	1	7	12	30	49	65	73	62	52	31	11	5	1		
Yukon	..	..	..	..	..	..	2	8	18	26	38	49	36	29	17	9	2	..		
Alaska	..	..	..	..	..	..	1	5	11	21	35	44	30	24	13	5	1	1		
Yunnan	..	..	..	..	..	1	2	8	16	32	41	57	44	29	17	8	1	2		
Kashmir	..	..	..	..	..	1	1	2	8	12	20	35	17	14	7	3	1	1		
Turkestan	..	..	..	..	..	1	..	2	4	8	17	27	19	7	3	1	1	..		
Total	..	..	..	..	..	6	32	94	188	312	414	528	410	314	190	88	29	9	2614	201 ± 0.1

very significant ($P < 0.001$). The American seeds are, therefore, certainly longer than the European ones.

It is not very easy to get mature but unopened anthers from herbarium material, but a number of anthers on specimens from both continents could, however, be measured (table 3). The anthers of the American material seem to be significantly longer than those of the European plants ($P < 0.001$).

Summing up the results of the morphological investigations, it should be pointed out that although many of the morphological characters of the Northern Bedstraw from North America were not found to differ from those examined in material from Europe, a number of minor characters are shown to be different. These differences are all listed in table 4. Although each of the distinguishing characters might be of little taxonomic value when not accompanying other characters, they are so strictly associated into two groups only that they must be regarded as being good specific differences, should they be followed by a barrier of hybrid sterility or incompatibility isolating both groups effectively from each other biologically. The possible occurrence of such a barrier might be indicated through the aid of geographical studies, but usually it has to be studied by means of the methods of cytology or cytogenetics.

CHOROLOGY

The specimens from Manitoba, Iceland and Scandinavia most closely studied morphologically were always found to differ in *all* the characters mentioned above. No individual was found to be intermediate or to have these characters mixed in any way. In order to check if this was only a coincidence, based perhaps on the isolation by distance, the authors studied the herbarium specimens available from other parts of the distribution area of the Northern Bedstraw. It then became evident that *all* the individuals from the North American continent from New England and Quebec in the east, to California and Alaska in the west, have the same combination of characters as have the specimens from Manitoba. Moreover, the few plants studied from eastern

TABLE 3.—Length of mature, unopened anthers (Measurements in 0.1 mm)

	15	16	17	18	19	20	21	22	23	24	25		
Iceland	1	2	2	8	17	29	13	11	3	1	1		
Sweden	1	1	..	4	8	19	9	3	1	..	..		
Caucasus	1	..	2	4	6	12	5	5	1	2	..		
Total	3	3	4	16	31	60	27	19	5	3	1		
New York	..	..	..	..	..	..	..	1	..	..	1		
Manitoba	..	..	..	..	..	..	1	1	..	1	3		
Kashmir	..	..	..	..	..	..	..	..	1	1	..		
Total	..	..	..	..	..	..	1	2	1	2	4		
	26	27	28	29	30	31	32	33	34	35	36	n	M ± m
Iceland	..	..	..	..	..	..	..	..	..	..	..		
Sweden	..	..	..	..	..	..	..	..	..	..	..		
Caucasus	..	..	..	..	..	..	..	..	..	..	..		
Total	..	..	..	..	..	..	..	..	..	..	..	172	20.0 ± 0.1
New York	2	4	8	19	6	5	2	1	..	1	..		
Manitoba	8	13	29	42	27	15	5	5	1	2	1		
Kashmir	2	4	7	15	5	5	2	..	1	1	..		
Total	12	21	44	76	38	25	9	6	2	4	1	248	29.1 ± 0.1

Asia and far into central Asia also, belong exclusively to this type. The localities from which material has been seen by the authors are marked with dots on the map in fig. 1, and the supposed distribution area of this type is shown by means of a stretch line.

Individuals from the Eurasiatic continent and islands from the localities marked with an open ring on the map in fig. 1, however, were found to belong to the same type of the combined characters given above as the Icelandic and Scandinavian specimens. The supposed approximate distribution of this type

TABLE 4.—Morphological differences between the Eurasiatic and the American-Asiatic types of the Northern Bedstraw

Character	Eurasiatic type	American-Asiatic type
Nodes:	Glabrous or with scattered hairs.	Clearly puberulent.
Bracts:	Circular-ovate, obtuse.	Oblong, acute.
Panicle:	Less divaricate.	More divaricate.
Width of corolla:	3.69 ± 0.001 mm.	4.69 ± 0.001 mm.
Color of petals:	Creamy.	Bright white.
Length of fruits:	1.71 ± 0.001 mm.	2.01 ± 0.001 mm.
Length of anthers:	2.0 ± 0.01 mm.	2.91 ± 0.01 mm.

Fig. 1.—The distribution areas of *Galium boreale* L. and *G. septentrionale* R. & S. Open rings and dotted line: *G. boreale*. Dots and stretch line: *G. septentrionale* 2.0 ± 0.01 mm.

is shown in fig. 1 by a dotted line. Although the areas in central Asia are somewhat obscure as yet, the few individuals from central Siberia where both types may be met with, were completely unmixed, thus indicating that hydridization between the two types does not occur there, or that it is extremely rare.

The firm linkage of the minute morphological characters observed to be different in these two types in question is a strong indication that they may belong to two taxa of a high rank. This indication is further strengthened by the differences in their geographical distribution as well as in the lack of intermediate types in the collections from the regions where both are found to grow almost together. Nevertheless, these indications alone are not strong enough to decide whether the types should be regarded as two different species or only as two major geographical races, or subspecies of the same species. All intraspecific categories are supposed to be completely interfertile, while different species are intersterile to a greater or lesser degree (cf. Du Rietz, 1930; Löve, 1951a; Rothmaler, 1944, 1950; Stebbins, 1950). The total lack of hybrids in the material from central Asia might seem to indicate the occurrence of some barrier of hybrid sterility or incompatibility. However, as the specimens studied from these regions are few and perhaps isolated by some geobotanical barriers unknown to the authors, this suggestion is of little taxonomical value. Therefore, the problem can only be solved on the basis of cytological or cytogenetical investigations.

CYTOLOGY

In fact, all sterility barriers may be classified into two groups, based upon their origin, and these groups have to be studied cytogenetically by different methods. The majority of species of Spermatophytes seems to have been formed by a gradual change in their genetical constitution and the arrangement of genes in their chromosomes, perhaps, following or followed by a geographical isolation or differentiation. Such species have been named gradual-ecospecies or g-ecospecies by biosystematists (Valentine, 1949, 1952). Their sterility barriers can only be studied cytogenetically after hybridization experiments, possibly in combination with genecological investigations, although morphological differences between their chromosomes might be dectectable directly after a rough cytological examination of their mitotic phases.

In about 30% (cf. Löve & Löve, 1949; Löve, 1951; Stebbins, 1938, 1950), of the higher plants, however, the sterility barriers between a new and an old species seem to have been formed suddenly through a change in the number of chromosomes. These revolutionary alterations may be arrived at on the basis of different types of autoploidy or alloploidy (cf. Löve & Löve, 1949; Stebbins, 1950), following catastrophic internal or external changes. However, they are always followed by a strong barrier of sterility, by some morphological distinction which grows stronger with time, as well as by some changes in the chorological or ecological reactions of the plant (cf. Löve & Löve, 1949; Löve, 1951a; Stebbins, 1950). Such species are named abrupt-ecospecies or a-ecospecies or even a-coenospecies by biosystematists (Valentine, 1949). Although a large group of these latter types have already been cytologically compared, there is no exception to the rule of sterility barriers between such types and their "parent" species, except perhaps where only some genically inert B-chromosomes have been duplicated (cf. Löve, 1943, 1951a). The a-ecospecies and a-coenospecies are the ideal subject for cytotaxonomical investigations, as the

difference in chromosome number alone will reveal the occurrence of a sterility barrier which in turn is one of the basic indications of their belonging to different species (cf. Löve, 1951a; Rothmaler, 1950; Lange, 1952).

A simple determination of the chromosome number of morphologically different types will immediately reveal their nature as a-ecospecies or a-coenospecies or not, but closer experimental studies are needed before it can be ascertained whether types with the same chromosome numbers, in spite of great morphological differences, are g-ecospecies or only intraspecific variations. In searching for sterility barriers it is, therefore, always highly recommendable to begin the studies by determining the chromosome numbers of the types in question. If they are found to differ, their biological isolation is ascertained. If not, closer genecological investigations should not be avoided in order to get fuller information on their cytogenetical as well as their taxonomical status.

There are several reports of chromosome numbers in the genus *Galium*, though the great majority of species described by taxonomists still remains cytologically unknown. It is deplorable, however, that far too many of the chromosome numbers published for different species of this critical genus have referred to the species in its widest sense only, probably due to the fact that the cytologist in question had only an unsatisfactory manual at his disposal. Frequently authors reporting chromosome counts from *Galium* also have omitted to give exact information regarding the region where their material was collected. Worse still, they seldom seem to have placed preserved specimens in any official herbarium. In such a critical genus as *Galium*, such an omission is a very serious one from the taxonomist's point of view and makes many of the results reached completely worthless (cf. Sterner, 1944). Some of the numbers published too, seem to be only guess-work from measurements of pollen grains (Ehrendorfer, 1950), a highly disputable method, and one not to be recommended even in less critical genera showing a much lower frequency of alloploidy.

Two different chromosome numbers have been reported for the species *G. boreale* s. lat. by different scientists. Homeyer (1932, 1935) listed the number $2n = 66$ chromosomes in material of unknown origin. Fagerlind (1937) counted the chromosomes of Swedish specimens and obtained $2n = 44$. Turesson (1938), however, confirmed the number published by Homeyer (l.c.) in material which had been collected in the province Shansi in northern China, while Poucques (1949) confirmed the number published by Fagerlind (l.c.) in French material. Böcher & Larsen (1950), however, reported that they had counted both the numbers, the tetraploid one, $2n = 44$, was typical in Danish and Icelandic material, while the hexaploid number, $2n = 66$, was counted in specimens from Alaska.

The present writers have studied the chromosome numbers of the Northern Bedstraw with long-haired fruits (var. *boreale*) as well as those of specimens with glabrous fruits (var. *hyssopifolium*) from the province Scania in southern Sweden. The chromosome number was exactly the tetraploid one, $2n = 44$ chromosomes, and the same number was also counted in material of typical var. *boreale* from several localities in Iceland.

Material of the American populations was fixed from the neighborhood of the University of Manitoba at Fort Garry, near Vivian, at Arborg, as well as from several localities near Delta at Lake Manitoba. Most of the plants had fruits with long hairs, some specimens from Fort Garry had glabrous fruits,

while two plants from near Vivian had fruits with appressed hairs. All these individuals were found to have precisely the hexaploid chromosome number $2n = 66$.

All the individuals with $2n = 44$ chromosomes studies by the present, as well as by previous writers, fall certainly within the distribution area of the taxon often referred to as the Eurasiatic type, characterized by glabrous or practically glabrous nodes, circular or ovate and obtuse bracts, creamy flowers somewhat smaller than in the so-called American type, with smaller fruits, and smaller anthers. The specimens with $2n = 66$ chromosomes, however, seem to fall within the boundaries of the other taxon with puberulent nodes, oblong and acute bracts, bright white flowers somewhat larger than in the other type, with bigger fruits and larger anthers. There is, however, one exception, the material studied by Homeyer (1932, 1935), the origin of which remains unknown. Should that material have come from the neighborhood of Kiel in Germany, where he performed his investigations, the hexaploid taxon would also occur in central Europe.

In order to check the accuracy of this last suggestion which would indicate that more than two chromosome numbers occur within the morphological type usually referred to as the Eurasiatic type, the writers studied herbarium material from the neighborhood of Kiel. It was found to be exactly of the same type as the Danish material, which is known to be tetraploid (Böcher & Larsen, 1950). Unfortunately, Homeyer does not seem to have conserved herbarium material of his plants, and as it was impossible to obtain any reliable information about this material, the writers asked his teacher, Professor Dr. GEORG TISCHLER in Kiel, for help in getting seed material from the neighborhood of Kiel. He sent us a large number of seeds which had been collected in the neighborhood of the city in the fall of 1951 by Dr. G. RAABE of the Botany Department of the University of Kiel. The seeds were germinated in Petri dishes in Winnipeg and fixed in the same way as the other material. As their chromosome number was found to be $2n = 44$, it is safe to conclude that the material studied by Homeyer (l.c.) of this species must have come from seed he obtained from foreign botanical gardens ("Die meisten der von mir untersuchten Pflanzen wurden aus Samen herangezogen, die ich im Austausch von anderen botanischen Garten erhielt," Homeyer, 1935, p. 238), and most probably from some of the gardens within the range of the so-called American type described above.

TAXONOMY

If a barrier of hybrid sterility or incompatibility related to morphological dissimilarities and differences in distribution is considered as a good indication that the two taxa should not be regarded as being intraspecific (cf. Lange, 1952), there is no doubt that the tetraploid and hexaploid types of the Northern Bedstraw should be kept as separate species. As Linnaeus (1753) gave northern Europe as the distribution area of the species *Galium boreale* L. it is quite evident that this species in its strict sense is identical with the tetraploid Eurasiatic plant described several times above. As the hexaploid taxon seems to be met with in North America and eastern and central Asia only, it should be given a species name different from the Eurasiatic one.

It was pointed out by Löve (1951a) that a large number of the so-called "intra-specific chromosome races" of geobotanists and cytogeneticists have previously been recognized as distinct species by classical taxonomists working

on the basis of morphological characters only, which have often been overlooked by more recent authors of manuals. A number of examples were drawn from the floras of northern Europe, but many more are known to exist in different cytotaxonomical papers. In the case of the hexaploid type of the Northern Bedstraw this seems to be equally true: it has been described as a separate species from North America at least on two different occasions.

In the year 1818 Roemer & Schultes subdivided the Linnaean *Galium boreale:* an entity in the strict sense as described by Linnaeus (1753) and the North American species *Galium septentrionale*. Their description was based upon that given in the flora by Pursh (1814), with the species known to occur in eastern North America, the Canadian lakes, as well as somewhere in Siberia. A year later Torrey (1819) separated the eastern North American material from the Linnaean species under the name *Galium strictum*. Although later authors, including Torrey (1824, 1843) himself, rejected the new species and continued to regard the American material as identical with the Linnaean species, there is no doubt that the morphological descriptions given by Roemer & Schultes (l.c.) as well as by Torrey (l.c.) apply only to the hexaploid material studied by the present writers. The name *G. strictum* Torr. must be rejected on the grounds of synonymy, while the name *G. septentrionale* R. & S. is the first validly published name for the hexaploid taxon hitherto referred to as the American type of the Northern Bedstraw.

As the intraspecific variations in hairiness of the fruit reported for both the species and mentioned in the introduction of the present paper, were described from the Eurasiatic species only, their names should not be applied to the American varieties without a new description. Before closer investigations into their real taxonomical value have been carried out, the present writers consider it inconvenient to give them any new Latin names. It might, however, be pointed out that although the typical variety of *G. borealis* certainly has villous-hirsute fruits, the types with glabrous or glabrate fruits are most probably the type variety of *G. septentrionale*, as that species was originally described as being from the area where these types are of most frequent occurrence.

DISCUSSION

Although the principles of the classification of biological entities have from early times aimed at getting as close as possible to the natural system, no general agreement has yet been reached (cf. Rothmaler, 1950; Lawrence, 1951). This is perhaps mainly due to a lack of experimental methods for studies on the relationship of taxa above the levels of species and genera.

As the early taxonomists did not even know experimental methods for analyzing the relationship of types of the lower categories, they had to depend upon morphological studies only, sometimes with the support of geographical observations. The concepts of the different taxonomical levels have, perhaps, changed somewhat from time to time (cf. Rothmaler, 1950; Gilmour, 1951). Nevertheless, those changes have not been so profound that most, if not all, recent definitions at the species level (cf. Babcock, 1947; Camp, 1944; Camp & Gilly, 1943; Clapham, 1949; Clausen, Keck & Hiesey, 1940, 1945; Danser, 1929; Darlington, 1940; Diver, 1940; Dobzhansky, 1937; Du Rietz, 1930; Ehrendorfer, 1950; Gleason, 1952; Goldschmidt, 1940; Gustafsson, 1947; Huxley, 1942; Just, 1944; Lange, 1952; Lawrence, 1951; Löve, 1951a; Mansfeld, 1948, 1949; Mayr, 1944; Nannfeldt, 1938; Rensch, 1929, 1938, 1947;

Rollins, 1952; Rothmaler, 1944, 1950; Simpson, 1944; Stebbins, 1950; Turesson, 1922, 1925, 1929, 1930, 1932, 1936, 1938; Turrill, 1940, 1949, 1950; Valentine, 1949, 1951; Wilmott, 1949; Zimmermann, 1931, 1949; etc.), regardless how elaborate they may be, still seem to be covered by the shorter and more concise one: "distincta propagatio ex semine," given already by Rajus (1686). It may well include the present demands for sterility barriers (cf. Lange, 1952) and morphological differences without any scholarly circumscription.

Without any knowledge of biological isolation mechanisms, the purely morphological methods of the classical taxonomists with the good floristic eye, were mostly so exact in separating the species from the intraspecific variations that the biosystematists have only been able to confirm the correctness of most of their work in classification. Sometimes, of course, the morphological methods failed, but there are many examples showing that even if authors of later manuals did not agree with the old taxonomists and regarded their species as intraspecific entities only, later biosystematical treatments have confirmed the original opinion in every detail (cf. Löve, 1951a). The species *Galium septentrionale* R. & S. is an excellent example thereof.

It has been demonstrated in the present paper that the Eurasiatic species *Galium boreale* L. and the American-Asiatic species *G. septentrionale* R. & S. are biologically isolated by a barrier of sterility caused by a difference in their respective number of chromosomes. It remains unknown, however, if they have been formed by auto- or alloploidy (cf. Stebbins, 1950), but nevertheless the hexaploid species is certainly an a-ecospecies as compared with the other. Still they have most of the major characteristics in common, while a strong linkage between many minor characters typical of each taxon seems to indicate a later genetical divergence or, perhaps, some qualitative differences from the beginning.

Without doubt, sterility barriers are of a much more taxonomical significance than are morphological differences, although distinctive characters are always of importance to the taxonomist in recognizing the different taxa. Some genera are characterized by clear and obvious morphological differences between their biologically isolated species, while species of other genera, as with many grasses and sedges, as well as the genus *Galium*, are distinguishable in minor differences only. Nevertheless, types of these genera with only a few and small morphological characteristics are just as good species as those of genera with great morphological differences, if they are biologically isolated from each other by means of a barrier of hybrid sterility or incompatibility. Or, in the words of the eminent Swedish taxonomist and geobotanist Samuelsson (1922): "Das unsere Augen den morphologischen Unterschied als ziemlich unbedeutend auffassen, hat unter solchen Umständen wenig zu sagen." Or, as the German taxonomist Rothmaler (1944) put it: "Vor allem muss man vermeiden, Heterogenes in eine systematische Einheit zusammenzuziehen. . . . Dazu kommt, dass ökologisch und soziologisch, systematisch und pflanzengeographisch nur mit den kleinsten Einheiten sicher gearbeitet werden kann." It does not help the progress of science to lump together different species in order to avoid work of a more exacting nature, and it is certainly always "a lesser evil to keep forms separated that are identical than to identify such as are distinct," as the prominent Swedish paleobotanist Halle (1913) once said in a discussion with his outstanding British colleague Seward.

It is a known fact that differences in chromosome number without exception reveal the occurrence of a more or less strong isolating barrier (cf. Valentine, 1949; Löve, 1951a). The number of chromosomes in a species in the strict sense is known to be one of the most constant of all its morphological and anatomical characteristics. Even small changes in that number will always result in changes in the external morphology and the physiology of the plant, as they directly affect the genes, which are located on the chromosomes themselves. Nevertheless, in a paper published as recently as 1951, the Austrian taxonomist Ehrendorfer (1951, cf. also 1949)—after determining one chromosome number in a critical group of *Galium* and deducting other numbers from it by pollen statistical studies only—speaks about the "grossen Labilität der Chromosomenzahlen und ihrem damit geringen diagnostischen Wert." Even Hultén (1951) seems to regard the chromosomes, their number, and their type, as inner-morphological differences of no systematic value, probably due to the same lack of acquaintanceship as Ehrendorfer (l.c.) with the results obtained from cytological investigations in more than 17,000 species of higher plants. All cytogeneticists and cytotaxonomists will, however, agree that the chromosome number itself is not a morphological character usable in the classification of plants in herbaria. As differences in the number of chromosomes are, however, always found to be followed by a biological isolation as well as by minor or major morphological differences, a detection of more than one chromosome number within a group previously regarded as a good taxonomical species should always be followed by a thorough search for morphological characteristics in order to obtain a more correct classification of these taxa. It should never be overlooked that the biologically isolated species in nature are the primary facts, our morphological classification of them the secondary thing only, and that there is no general rule determining that all good species should be separable by very conspicuous characters only.

The differentiation of *G. boreale* and *G. septentrionale* by a few minor morphological characters only might, perhaps, seem indicative of their being of more recent origin. This, however, is not necessarily so. Several biologists have pointed out that in advanced types especially the rate of evolutionary changes in morphological characters may be very slow (cf. Simpson, 1944; Small, 1949; Stebbins, 1947, 1949, 1950), so that some species might even have remained practically unchanged for millions of years. This seems to be the case in many polyploid complexes (cf. Löve, 1943, 1951b; Löve & Löve, 1951; Stebbins, 1949). The two taxa of *Galium* considered here are both polyploid and advanced, and it is remarkable how little their intraspecific variations are, in spite of their vast areas of distribution.

The true age of the hexaploid species is not easily deducible from its present distribution area. It might seem to be rather uncertain if it reached Gaspé in eastern North America before the last glaciation (cf. Fernald, 1925; Wynne-Edwards, 1937; Scoggan, 1950), since its localities on this peninsula are not isolated from its southern populations. However, the fact that only one of the three varieties known occurs in Gaspé and vicinity, might perhaps support the view that it is a glacial survivor there. The tetraploid species, however, certainly belongs to the glacial survivors in Scandinavia (cf. Hultén, 1950), so that it must have reached western Europe at least prior to the last glaciation. It apparently does not disperse over sea, as it is unknown on the island of Grimsey just off the northern Icelandic coast (cf. Jones, 1937; Löve, 1950),

and it seems reasonable to regard it as quite unlikely that it has been borne by winds, birds, or sea currents from Europe to Iceland (for references, cf. Löve & Löve, 1947). If the last assumption holds true the species must have spread from the European continent to Iceland prior to its disconnection with other countries. The exact time of that separation is not yet known, but all geologists seem to agree in that it must have occurred in the Tertiary Period and most of them regard the late Miocene as the most probable time (Thoroddsen, 1914; Schuller & Schuchert, 1917; Högbom, 1938; Wegmann, 1948; cf. also Löve, 1951b; Löve & Löve, 1951). In that case the species *G. boreale* must have reached not only western Europe but even Iceland long before the Pleistocene glaciations commenced.

The indications presented above on the age of the species *G. boreale* and *G. septentrionale* are not definite and should be regarded as merely speculative as they are based on the much too fragmentary knowledge of the age of the North Atlantic islands. Nevertheless, it seems quite evident that both taxa should be regarded as old species with a slow rate of evolution after creation by polyploidy long ago somewhere in Central Asia.

Acknowledgement.—The authors wish to thank the National Research Council of Canada for its partial financial support of this investigation.

SUMMARY

The collective circumpolar species *Galium boreale* has been divided into a few varieties based on the hairiness of the fruits, as well as into two different species not recognized by authors of recent manuals.

It is shown that the two species concerned differ in some minor morphological characters such as the puberulence of the nodes, the form of the bracts, the type of the panicle, the size of the flower, anthers, and seeds, and the color of the corolla. These characters are always found to be strictly associated and do not mix freely.

The geographical distribution of both the taxa is found to differ considerably since one of them occurs all over northern and central Europe west to Iceland, as well as eastwards in central Siberia, while the other grows in central Asia and eastwards to Gaspé and Nova Scotia in North America.

Cytological investigations showed that the Eurasiatic taxon is a tetraploid with $2n = 44$ chromosomes, while the American-Asiatic one is a hexaploid with $2n = 66$ chromosomes.

Taxonomical studies of the types revealed that the tetraploid is identical with the Linnaean species *G. boreale* in its strict sense as described from northern Europe, while the hexaploid species should be named *G. septentrionale* according to a description of eastern North American material given by Roemer & Schultes.

Both species are assumed to be of ancient origin.

REFERENCES

BABCOCK, E. B. 1947—The genus *Crepis*. Part 1. The taxonomy, phylogeny, distribution, and evolution of *Crepis*. Univ. Calif. Publ. Bot. 21:1-197.

BECK VON MANNAGETTA, G. 1893—Flora von Niederösterreich. 2. Wien.

BIGELOW, J. 1814—Florula Bostoniensis. Boston.

BÖCHER, T. W. 1938—Biological distributional types in the flora of Greenland. Medd. om Grönl. 106, 2:1-339.

CAMP, W. H. 1944—A preliminary consideration of the biosystematy of *Oxycoccus*. Bull. Torrey Club 71:426-437.

——— & GILLY, C. L. 1943—The structure and origin of species. Brittonia 4:323-385.

CLAPHAM, A. R. 1949—Ecology and critical groups. In Wilmott (ed.): British flowering plants and modern systematic methods, 1949:23-27.
CLAUSEN, J., D. D. KECK & W. M. HIESEY 1940—Experimental studies on the nature of species. 1. Effect of varied environments on western North American plants. Carnegie Inst. Wash. Publ. 520:1-452.
———, ——— & ———1945—Experimental studies on the nature of species. 2. Plant evolution through amphiploidy and autoploidy, with examples from the Madiinae. Ibid. 564:1-174.
DAHL, O. 1937—Floraen i Finnmark Fylke. Nyt Mag. f. Naturv. 69:1-430.
DANSER, B. H. 1929—Über die Begriffe Komparium, Kommiskuum und Konvivium und über die Entstehungsweise der Konvivien. Genetica 11:399-450.
DARLINGTON, C. D. 1940—Taxonomic species and genetic systems. The New Systematist:137-160.
——— & L. F. LA COUR 1942—The handling of chromosomes. London.
DARLINGTON, W. 1837—Flora Cestrica: An attempt to enumerate and describe the flowering and filicoid plants of Chester County in the state of Pennsylvania, etc. Westchester.
DECANDOLLE, A. P. 1830—Prodromus systematis naturalis regni vegetabilis. Pars quarta. Paris.
DIVER, C. 1940—The problem of closely related species living in the same area. The New Systematics:303-328.
DOBZHANSKY, TH. 1937—Genetics and the origin of species. New York.
DRUCE, G. C. 1930—Hayward's botanist's pocket-book, 19th ed. London.
DU RIETZ, G. E. 1930—The fundamental units of biological taxonomy. Svensk Bot. Tidskr. 24:333-428.
EHRENDORFER, F. 1949—Zur Phylogenie der Gattung *Galium*. 1. Polyploidie und geographisch-ökologische Einheiten in der Gruppe des *Galium pumilum* Murr. (Sekt. *Leptogalium* Lange sensu Rouy) im österreichischen Alpenraum. Österr. Bot. Zeitschr. 96:109-138.
———1950—Moderne Floristik und Beziehungen zu Systematik, Pflanzengeographie, Genetik und Zytologie. Arb. Bot. Stat. Hallstadt, Nr. 103.
———1951—Rassengliederung, Variabilitätszentren und geographische Merkmalsprogressionen als Ausdruck der raum-zeitlichen Entfaltung des Formenkreises *Galium incanum* S.S. (Eine Monographie). (Zur Phylogenie der Gattung *Galium*. 2.) Österr. Bot. Zeitschr. 98:427-490.
FAGERLIND, F. 1937—Embryologische, zytologische und bestäubungs-experimentelle Studien in der Familie Rubiaceae, nebst Bemerkungen über einiger Polyploiditätsprobleme. Acta Horti Bergiani 11:195-470.
FERNALD, M. L. 1925—Persistence of plants in unglaciated areas of boreal America. Amer. Acad. Arts & Sci. Mem. 15:241-342.
———1928—The varieties of *Galium boreale*. Rhodora 30:106-107.
———1950—Gray's manual of botany, 8th ed. New York.
GILMOUR, J. S. L. 1951—The development of taxonomic theory since 1851. Nature 168:400-402.
GLEASON, H. A. 1951—Some fundamental concepts in taxonomy. Phytologia 4:1-20.
GOLDSCHMIDT, R. 1940—The material basis of evolution. New Haven.
GRENIER, J. C. M. & D. A. GODRON 1947-52—Flore de France. Besançon.
GUSTAFSSON, A. 1947—Apomixis in higher plants. Part 3. Biotype and species formation. Acta Univ. Lund. N.S. II, 43, 12:183-370.
HALLE, T. G. 1913—Some remarks on the classification of fossil plants. Geol. Fören. Förhandl. 35.
HÖGBOM, A. G. 1938—Die Atlantislitteratur unserer Zeit. Betrachtungen eines Geologen. Bull. Geol. Inst. Uppsala 28.
HOMEYER, H. 1932—Zur Zytologie der Rubiaceen. Planta 18:640.
———1935—Beiträge zur Kenntnis der Zytologie und Systematik der Rubiaceen. Bot. Jahrb. 67:238-263.
HOOKER, W. J. 1940—Flora Boreali-Americana; or the botany of the northern parts of British America, Vol. 2. London.
HULTÉN, E. 1949—Flora of Alaska and Yukon, 9. Acta Univ. Lund, N.S. II, 45, 1:1345-1481.
———1950—Atlas över växternas utbredning i Norden. Stockholm.

———1951—Recension: Flora SSSR. (Flora Sojusa Sovjetskich Socialistitcheskich Respublik), del 1-14 (Pteridophyta-Vitaceae). Svensk Bot. Tidskr. 45:258-265.
HUXLEY, J. 1942—Evolution, the modern synthesis. London.
JONES, E. W. 1937—The vegetation of Grímsey, Iceland. Journ. Ecol. 25:222-253.
JUST, TH. 1944—The rates of evolutionary processes. Proc. Indiana Acad. Sci. 53:14-27.
KOTILAINEN, M. J. 1951—Über die Verbreitung der meso-eutrophen Moorpflanzen in Nordfinnland. Ann. Acad. Sci. Fenn. A, IV, 17:1-162.
LANGE, M. 1952—Species concept in the genus *Coprinus*. Dansk Bot. Arkiv 14, 6:1-164.
LAWRENCE, G. H. M. 1951—Taxonomy of vascular plants. New York.
LID, J. 1944—Norsk flora. Oslo.
LINNAEUS, C. 1753—Species plantarum. Holmiae.
LÖVE, Á. 1943—Cytogenetic studies on *Rumex* subgenus *Acetosella*. Hereditas 30:1-136.
———1945—Islenzkar jurtir. Kaupmannahöfn.
———1950—Some innovations and nomenclatural suggestions in the Icelandic flora. Bot. Notiser 1950:24-60.
———1951a—Taxonomical evaluation of polyploids. Caryologia 3:263-284.
———1951b—The Icelandic type of *Glyceria fluitans*. Bot. Notiser 1950:229-240.
——— & D. LÖVE 1947—Studies on the origin of the Icelandic flora. I. Cyto-ecological investigations on *Cakile*. Icel. Univ. Inst. Appl. Sci., Dept. of Agric. Rep. B, 2:1-29.
——— & ———1949—The geobotanical significance of polyploidy. I. Polyploidy and latitude. Portug. Acta Biol. (A), R. B. GOLDSCHMIDT Vol.:273-352.
——— & ———1951—Studies on the origin of the Icelandic flora. II. Saxifragaceae. Svensk Bot. Tidskr. 45:368-399.
MANSFELD, R. 1948—Über den Artbegriff in der systematische Botanik. Biol. Zentralbl. 67:320-331.
———1949—Die Technik der wissenschaftlichen Pflanzenbenennung. Berlin.
MAYR, E. 1944—Systematics and the origin of species. New York.
NANNFELDT, J. A. 1938—*Poa maroccana* Nannf. n. sp. and *P. rivulorum* Maire & Trabut, two more tetraploids of sects. *Ochlopoa* A. & Gr., and some additional notes on *Ochlopoa*. Svensk Bot. Tidskr. 32:295-321.
NEWTON, W. C. F. & C. D. DARLINGTON 1929—Meiosis in polyploids. 1. Journ. of Genet. 21:1-16.
NORDHAGEN, R. 1940—Norsk flora. Oslo.
OSTENFELD, C. H. 1926—The flora of Greenland and its origin. Kgl. Danske Vidensk. Selsk. Biol. Medd. 6, 3:1-71.
POLUNIN, N. 1940—Botany of the Canadian Eastern Arctic. Part 1. Pteridophyta and Spermatophyta. Nat. Mus. Canada, Bull. 92:1-408.
PORSILD, A. E. 1951—Botany of southeastern Yukon adjacent to the Canol Road. Ibid. 121:1-400.
POUCQUES, M.-L. DE. 1949—Recherches caryologiques sur les Rubiales. Rev. Gén. Bot. 56:5-27, 74-138, 172-188.
PURSH, F. 1814—Flora Americae Septentrionalis: or a systematic arrangement and description of the plants of North America. 2 vols. London.
RAJUS, J. 1686—Historia plantarum, etc. Londini.
RASMUSSEN, R. 1936—Föroya flóra. Tórshavn.
RAYMOND, M. 1950—Esquisse phytogéographique du Québec. Mém. Jard. Bot. Montréal 5:1-147.
RENSCH, B. 1929—Das Prinzip geographischer Rassenkreise und das Problem der Artbildung. Berlin.
———1938—Some problems of geographical variation and species formation. Linn. Soc. London, Proc. Session 150:275-286.
———1947—Neuere Probleme der Abstammungslehre. Stuttgart.
ROEMER, J. J. & J. A. SCHULTES 1818—Caroli a Linne Systema vegetabilium. Stuttgardtiae.
ROLAND, A. E. 1944—The flora of Nova Scotia. Proc. Nova Scotia Inst. Sci. 21, 3:1-552.
ROLLINS R. C. 1952—Taxonomy today and tomorrow. Rhodora 54:1-19.
ROTHMALER, W. 1944—Systematische Einheiten in der Botanik. Feddes Repert. 54:1-22.
———1950—Allgemeine Taxonomie und Chorologie der Pflanzen. Jena.
SAMUELSSON, G. 1922—Zur Kenntnis der Schweizer flora. Vierteljahrschr. d. Naturforsch. Ges. Zürich, 67.

SCHULLER, R. & C. SCHUCHERT 1917—Atlantis, the "lost" continent. A review of Termier's evidence. The Geogr. Review 3.
SCHUMANN, K. 1891—Rubiaceae. In Engler-Prantl, Die natürlichen Pflanzenfamilien etc., IV, 4:1-156.
SCOGGAN, H. J. 1950—The flora of Bic and the Gaspé Peninsula, Québec. Nat. Mus. Canada, Bull. 115:1-399.
SIMPSON, G. G. 1944—Tempo and mode in evolution. New York.
SMALL, J. 1949—Quantitative evolution 17. The shape and pattern of evolution. Phyton 1:269-281.
STEBBINS, G. L. 1947—Evidence on rates of evolution from the distribution of existing and fossil plant species. Ecol. Monogr. 17:149-158.
———1949—Rates of evolution in plants. Genetics, Paleontology and Evolution:229-242.
———1950—Variation and evolution in plants. New York.
STEFÁNSSON, S. & S. STEINDÓRSSON 1948—Flóra Islands, 3. útg. Akureyri.
STERNER, R. 1944—*Galium pumilum* Murr. i nordvästra Evropa. Acta Horti Gothoburg. 15:187-233.
STEVENS, O. A. 1950—Handbook of North Dakota plants. Fargo.
THORODDSEN, TH. 1914—An account of the physical geography of Iceland with special reference to the plant life. Bot. of Iceland 1:187-343.
TORREY, J. 1819—Catalogue of plants growing within thirty miles of the city of New York. Albany.
———1824—A flora of the northern and middle sections of the United States, etc. New York.
———1843—A flora of the State of New York, etc. Vol. 1. Albany.
TURESSON, G. 1922—The genotypical response of the plant species to the habitat. Hereditas 3:211-350.
———1925—The plant species in relation to habitat and climate. Ibid. 6:147-236.
———1929—Zur Natur und Begränzung der Arteinheiten. Ibid. 12:323-334.
———1930—Genecological units and their classificatory value. Svensk Bot. Tidskr. 24:511-518.
———1932—Die Genzentrumtheorie und das Entwicklungszentrum der Pflanzenart. Kungl. Fysiogr. Sällsk. i Lund Förhandl. 2:76-86.
———1936—Die Bedeutung der Rassenökologie für die Systematik und Geographie der Pflanzen. Feddes Repert., Beih. 41:15-37.
———1938—Chromosome stability in Linnean species. Ann. Agric. Coll. Sweden 5:405-416.
TURRILL, W. B. 1940—Experimental and synthetic plant taxonomy. The New Systematics:47-71.
———1949—Chromosome numbers and taxonomy. Proc. Linn. Soc. London 161:119-125.
———1950—Modern trends in the classification of plants. Nature 166:384-385.
VALENTINE, D. H. 1949—The units of experimental taxonomy. Acta Biotheoretica 9:75-88.
———1951—Geographical distribution and isolation in some British ecospecies. In Lousley (ed.): The study of the distribution of British plants, 1951:82-90.
———1952—Studies on British primulas. 3. Hybridization between *Primula elatior* (L.) Hill and *P. veris* L. The New Phytol. 50:383-399.
WEBB, D. A. 1943—An Irish flora. Dundalk.
WEGMANN, C. E. 1948—Geological tests of the hypothesis of continental drift in the Arctic regions. Medd. om Grönl. 144, 7:1-48.
WILLIS, J. C. 1949—The birth and spread of plants. Boissiera 8:1-561.
WILMOTT, A. J. 1949—Intraspecific categories of variations. In Wilmott (ed.): British flowering plants and modern systematic methods, 1949:28-45.
WOMBLE, W. H. 1951—Differential systematics. Science 114:315-332.
WYNNE-EDWARDS, V. C. 1937—Isolated Arctic-alpine floras in eastern North America: a discussion of their glacial and recent history. Roy. Soc. Canada, Transact. III (5) 31:1-26.
ZIMMERMANN, W. 1931—Arbeitsweise der botanischen Phylogenetik. Abderhalden: Handb. Biol. Arbeitsmeth. IX, 3:941-1053.
———1949—Geschichte der Pflanzen. Stuttgart.

An Electron Microscope Study of Protozoan Flagella[1]

Harley P. Brown and Avalee Cox[2,3]
University of Oklahoma, Norman

Protozoan flagella are organelles which have been studied extensively; nevertheless, many structural details remain controversial. Because of the limited power of resolution of the light microscope, little of the finer structure of the flagellum was revealed before the advent of the electron microscope. That the organelle is composed of a central axial core (axoneme) and an enveloping sheath is rather generally accepted.

Early in the present century, flagella of *Euglena* and *Chilomonas* were shown to be separable into fibrils when subjected to pressure (Dellinger, 1909). Other workers (e.g., Korschikov, 1923) pointed out that fibrils occur within the axonemes of various flagellates. More recently, electron micrographs have demonstrated the presence of a number of fibrils within the axoneme—usually nine to twelve (Schmitt, Hall, and Jakus, 1943; Pitelka, 1949; Houwink, 1951). In the euglenoids, these fibrils appear to be arranged in two parallel bundles (Brown, 1945; Pitelka, 1949); however, Pringsheim and Hovasse (1950) interpret this appearance as being due to the ribbon-like structure of the flagellum. Manton and Clarke (1951) present electron micrographs which clearly demonstrate the presence of eleven fibrils in the cilium or flagellum of a fern spermatozoid.

The nature of the sheath is more difficult to determine. Apparently it consists of a semi-liquid substance enclosed by a membrane (Pitelka, 1949), and may include fibrils which encircle the axoneme in a helix (Brown, 1945). However, Houwink (1951) has demonstrated that in *Euglena* the membrane, if present, is very delicate, and that the transverse striations in the *Euglena* flagellum are not due to the presence of a helical fiber.

The functions of the flagellar components are unknown. Some authors (e.g., Pitelka, 1949) assume the axoneme to be the contractile portion, while others (e.g., Hyman, 1940; Houwink, 1951) consider the axoneme merely an elastic supporting structure, and contend that the sheath contains the contractile elements. The excellent electron micrographs of Houwink depict transverse striations in the sheath of the *Euglena* flagellum which are strikingly similar to the striations of skeletal muscle.

Most investigators who have made careful microscopic studies of such organisms as the phytomonads (*Chlamydomonas, Sphaerella,* etc.) have observed a delicate terminal filament (e.g., Kunstler, 1889; Loeffler, 1889; Fischer, 1894; Gerloff, 1940; Owen, 1949). Pitelka (1949) examined the flagella of *Sphaerella* by means of the electron microscope, but observed no

---

1 Based largely upon a thesis submitted by Miss Cox to the University of Oklahoma in partial fulfillment of the requirements for the degree of Master of Science.

2 The present address of Miss Cox is: Department of Biology, State A. & M. College, Magnolia, Arkansas.

3 We are indebted to Mr. Harold Gandy of the University of Oklahoma Department of Physics, who operated the electron microscope, and to the University of Oklahoma Research Institute for use of the instrument.

lash tips. Perhaps her use of the centrifuge in concentrating the organisms resulted in loss of the flagellar tips.

Many workers have detected delicate, hairlike appendages projecting laterally from flagella (e.g., Loeffler, 1889; Fischer, 1894; Petersen, 1929; Deflandre, 1934; Vlk, 1938; Brown, 1945; Pitelka, 1949; Houwink, 1951). Among the names applied to these appendages are *flimmer* (Fischer, 1894) and *mastigonemes* (Deflandre, 1945). The precise nature of the flimmer and their mode of origin have been subjects of considerable speculation. Pitelka considers them frayed fibrils from the sheath (at least in euglenoids), whereas others (e.g., Brown, 1945; Owen, 1947) have suggested that they might arise by extrusion and subsequent coagulation of protoplasm from the sheath. Controversy still exists as to whether flimmer are naturally-occurring structures upon living flagella or merely artifacts resulting from flagellar disintegration. Owen (1947) asserts that they are artifacts. Furthermore, he states that they cannot be demonstrated in specimens which are killed in satisfactory fixing reagents which harden protoplasmic structures in normal positions. Thus, using his modification of Gelei's fixative (Gelei, 1926), he was unable to demonstrate the presence of flimmer upon flagella of species which exhibit them when prepared according to Loeffler's (1889) method. On the basis of his conclusions, Owen discarded the use of flimmer for taxonomic purposes, and attempted to differentiate flagellar types according to the nature of the sheath.

The investigations upon which this paper is based were undertaken with the hope of clarifying some of the difficulties indicated above; e.g., reconciling the results of Owen with those of other recent workers.

MATERIALS AND METHODS

The flagellates used in this study were *Ochromonas variabilis* Meyer, *Astasia klebsii* Lemmermann, and *Chilomonas* sp. (probably *C. paramecium*). Cultures of the first two species were obtained from Professor W. J. Kostir of Ohio State University; the *Chilomonas* culture was acquired from Carolina Biological Supply House at Elon College, North Carolina.

No flagellum was photographed unless it was attached to an organism, except where it was obvious that a portion of the flagellum had been pulled away from the cell body by the rupturing of the supporting film, and where both the body with the attached basal part of the flagellum and the detached distal segment could be found.

For *Chilomonas* a culture medium of egg yolk (Kirby, 1947) seemed preferable, although boiled wheat was used successfully. Boiled wheat was also employed in culturing *Astasia*. In both instances, seven grains of wheat were boiled in sixty-five milliliters of pond water. One or two cubic millimeters of dried milk, boiled for five minutes in sixty-five milliliters of pond water, provided a favorable medium for *Ochromonas*. Cotton-plugged Erlenmeyer flasks (125 ml) served as culture vessels.

A supporting film for the organisms was prepared by a modification of techniques described by Brown (1945) and the Radio Corporation of America (1946).

The flagellates were prepared for electron microscope examination by two techniques which were identical except for the fixative. Organisms were taken from cultures in which they were abundant. Concentration by centrifuging

was avoided, since this would subject delicate structures to forces which might distort or destroy them. Technique 1 involved use of a 2% aqueous solution of osmic acid as a fixative. Technique 2 employed Owen's fixative (Owen, 1947: 10 parts of 2% osmic acid, 1 part of 20% formalin).

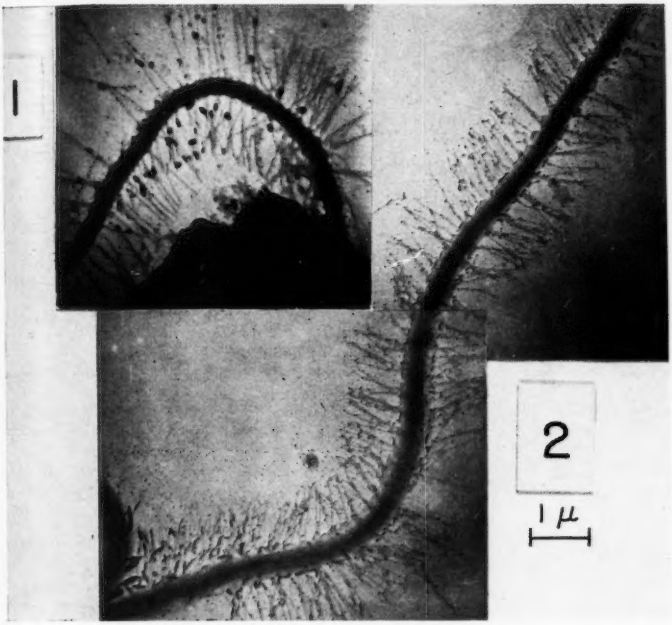
The electron microscope employed was an RCA Universal Model, type EMU. The operating voltage was about fifty kilovolts. A filament oscillator current of approximately one tenth to two tenths of a microampere was used; that of one tenth microampere yielded best results, with little or no rupturing of the collodion film. The reduced intensity of the electron beam necessitated a relatively long time of exposure (ten to fifteen seconds). All micrographs were made at an initial magnification of seven thousand diameters. The instrument had been calibrated by Miller (1949).

RESULTS AND DISCUSSION

Ochromonas variabilis.—*Ochromonas* is a small chrysomonad having two flagella of unequal length. This study, which is concerned chiefly with the long flagellum, is based upon thirty-seven micrographs of sixteen specimens.

In fig. 2, the flagellum appears to be comprised of a relatively dense axoneme enclosed by a less dense sheath. In all other micrographs of *Ochromonas* flagella, only the axoneme is apparent. Perhaps the sheath has become appressed to the axoneme in these other preparations. This seems more likely than that the sheath has been destroyed during preparation of the specimens. The difference in the appearance of the flagella shown in figs. 1 and 2, for example, may be due to a difference in drying conditions. Certain drying conditions might result in relatively greater movement of sheath substance toward the axoneme, causing the substance to adhere to or aggregate around the axoneme. In Pitelka's (1949) plates 41a, 42, and in Foster, Baylor, Meinkoth and Clark's (1947) plate I, fig. 2, there is a region of lesser density along the sides of the flagellum. This condition, which is similar to that seen in our figs. 1 and 3, might also be traced to the withdrawal of sheath material toward the axoneme. Another possible explanation for the fact that a sheath is evident only in fig. 2 might lie in swelling of the flagellar substance beneath a membrane or sheath which is closely appressed to the axoneme in the normal flagellum.

A comparison of the micrographs shows that the axoneme in fig. 4 is denser and more slender than in the other figures. This difference is probably due to the use of Owen's fixative in the preparation of fig. 4, whereas osmic acid alone was employed in all the other preparations. This micrograph is of considerable interest, since it shows the result of utilizing the fixative with which Owen (1947) was unable to demonstrate flimmer. He considered flimmer to be artifacts which are observed only after dry fixation or osmic acid fixation. It was his opinion that osmic acid-formalin is preferable to osmic acid, since the former apparently preserves the actual structure of the flagellum by coagulating protoplasm rapidly. If the almost complete absence of foreign particles (compare figs. 1 and 4) is characteristic of specimens prepared with Owen's reagent, it is unlikely that Owen could have observed flimmer with the limited resolving power of the light microscope. Even with a thin layer of stained mordant, the flimmer would probably be no more than 0.1 μ in diameter—hence below the critical level. However, with particles deposited upon the flimmer, as in fig. 2, or with tufts of flimmer sticking to-



Figs. 1, 2.—1. Long flagellum and part of cell body of *Ochromonas*. Note flimmer and fibrillar appearance of axoneme or core. Technique 1; 2. Long flagellum of *Ochromonas* showing sheath, axoneme, and flimmer. Short flagellum, without flimmer, barely shows at lower left. Technique 1.

gether, the dimensions might well be within the resolvable range of the ordinary microscope, and thus demonstrable either by the nigrosin technique of Deflandre (1923) or by the Loeffler (1889) technique employed by most workers. Nevertheless, it should be pointed out that flimmer may be detectable even when their individual diameters render them unresolvable. Thus, most flimmer observed with the light microscope are blurred and indistinct, frequently consisting of groups of fibrils. Owen figures a *Mallomonas* flagellum (Owen, 1949: plate 3, fig. 3) which certainly suggests clumped flimmer, although he states that they cannot be demonstrated by his fixation method.

Comparison of the density and width of the flagella shown in figs. 1-4 suggests, further, that the use of osmic acid-formalin as a fixative, instead of plain osmic acid, results in either less swelling or greater shrinkage.

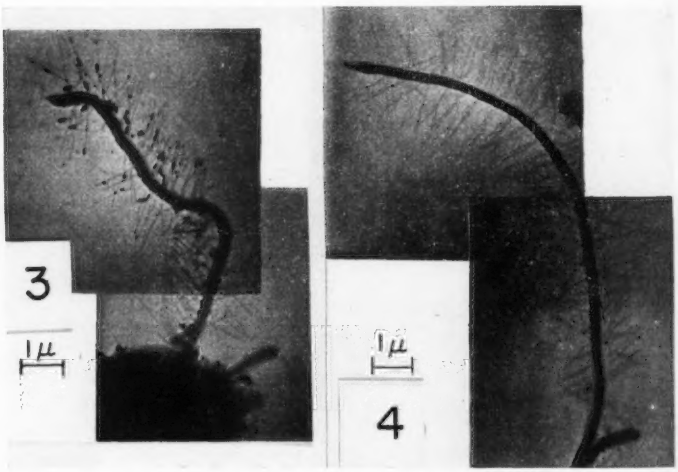
Figs. 1-3 suggest the fibrillar nature of the axoneme, although elongate particles of foreign materials deposited upon the surface of the flagellum might be responsible for the semblance of longitudinally oriented fibrils in some parts of fig. 2.

All of the micrographs of *Ochromonas* show flimmer on both sides of the long flagellum. These flimmer average an estimated 0.02-0.04 μ (200-400 Ångstrom units) in diameter and about 1.5 μ in length. They are generally

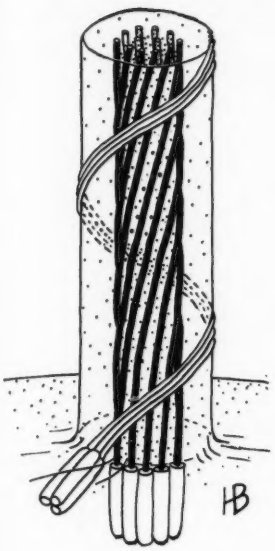
straight, and give the impression of relative rigidity, unlike euglenoid flimmer. Many of the flimmer occur in tufts of two or three filaments, the tufts arising from protuberances along the surface of the flagellum or the axoneme. Similar regularly-spaced bumps along the flagellum of *Ochromonas* are noticeable in Brown's (1945) plates 11 and 12, and were interpreted as being due to a helical fibril encircling the axoneme. Upon the basis of these appearances, it is tempting to speculate concerning the detailed structure of the flagellum. One line of interpretation and speculation is as follows:

The basal granule, from which the axoneme develops, is composed of a cluster of about ten or twelve "fibril factories" (fig. 5). Each of these "factories" consists of a number of enzyme molecules capable of producing elongate protein micelles or fibrils. As the fibrils are extruded toward the cell membrane, the latter is pushed outward, and, together with a thin layer of cytoplasm, forms the sheath which encloses the fibrillar core. A smaller "fibril factory," associated with the cell membrane at the base of the flagellum, extrudes a smaller bundle of fibrils—let us say a bundle of three. This "factory," being closer to the cell membrane and thus having greater access to oxygen diffusing inward, has a higher metabolic rate than the basal granule, and extrudes its fibrils somewhat more rapidly than does the basal granule. This difference in growth rate results in a coiling of the superficial fibrils about the axoneme fibrils, and produces the helical fiber of the sheath. Under certain conditions, the component fibrils fray into individual micelles which commonly remain attached at one end (fig. 6). These are the flimmer. In some places along the flagellum, all three of the fibrils or micelles of the helix fray or spring loose from the sheath, forming tufts of three flimmer. Elsewhere on the flagellum, only one or two fibrils may spring free. If the flagellum shrinks, as during drying, the sheath membrane becomes appressed to the axoneme (fig. 6). Thus, in silhouette, as in electron micrographs (e.g., fig. 4), bumps appear to occur along the edges of the axoneme wherever the helical fiber of the sheath comes around the axoneme, and the flimmer arise from these bumps.

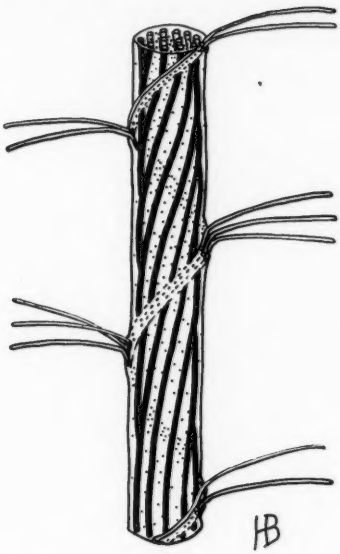
Although the above description is purely hypothetical, we might mention



Figs. 3, 4.—3. Flagella and part of cell body of *Ochromonas*. Flimmer occur only upon the long flagellum. Technique 1; 4. Flagella of *Ochromonas*. Note slimmer and denser flagella (as compared with figs. 1-3), and absence of particles adhering to flimmer. Clusters of flimmer arise from bumps along the flagellum. Technique 2.



5



6

Figs. 5, 6.—5. Diagram illustrating hypothetical structure of flagellar base where fibrils of axoneme and sheath are formed; 6. Diagram illustrating hypothetical mode of flimmer formation by fraying of fibrils from sheath.

a few of the facts suggesting such an interpretation, aside from appearance alone. The width of the flimmer (ca. 0.03 μ) is within the size range of the width of the axial fibrils (0.025-0.04 μ as cited for *Trichonympha* flagella by Schmitt, Hall, and Jakus, 1943; 0.035 μ as cited for *Euglema* by Houwink, 1951). The length of the flimmer (e.g., fig. 2) is just right for such an interpretation, being such that they could wrap half way around the flagellum, at approximately the helical pitch indicated in the diagrams. It is not uncommon for fibrous proteins (e.g., myosin, fibrin, collagen) to form fibrils of these dimensions. Pertinent discussions of such fibrils are presented by Schmitt (1944), Hall, Jakus and Schmitt (1946), De Robertis and Schmitt (1948), and Rozsa, Szent-Györgyi and Wyckoff (1950).

Even though this should be the correct interpretation of the nature and origin of flimmer, it provides no obvious clue as to whether the flimmer occur upon the intact living flagellum. Electron micrographs are unlikely to answer that question, although they have shown us that flimmer occur upon even those flagella prepared by Owen's method. Possibly a rapid-freeze drying technique (Drummond, 1950; Mendelow and Hamilton, 1950) might yield further evidence upon this matter.

Owen (1947) states that organisms like *Ochromonas* have a flagellum

"which does not show a distinct axoneme or has one much reduced in proportion to the plasma in which it is embedded." Our micrographs suggest the opposite, namely, that the sheath, if anything, is the "reduced" component. Nor do we feel that our micrographs corroborate Owen's statement: "In no case have the arrangement of flimmers been found to be constant." Instead we consider their constancy rather striking. Owen used only the light microscope.

A point which we wish to mention, although it has little direct bearing on the present discussion, is that comparison of our micrographs and those of several other authors with those of Brown (1945) suggests that the method of calibration employed by Brown was inaccurate. The 1 μ scales shown in his plates are probably about 3 times longer than they should be. Perhaps Professor Prebus, whose method Brown utilized, had misremembered one detail: perhaps it was the distance between adjacent holes of a single row on the diatom test which averaged $^1/_3$ μ rather than the distance between the rows. (See Brown, 1945, plate 1A.) This, apparently, would correspond closely to the calibrations of other authors.

Chilomonas sp.—In the study of *Chilomonas*, thirty-five micrographs were made of ten different specimens. Each of the two unequal flagella exhibits a relatively dense axoneme and a less dense sheath, both of which are well-defined (figs. 8, 9). The width of the sheath, at times greater on one side than on the other, diminishes toward the distal end of the flagellum. There is a faint indication of diagonal lines (or cross-striations?) in the sheath (fig. 8) similar to those discussed and figured by Houwink (1951).

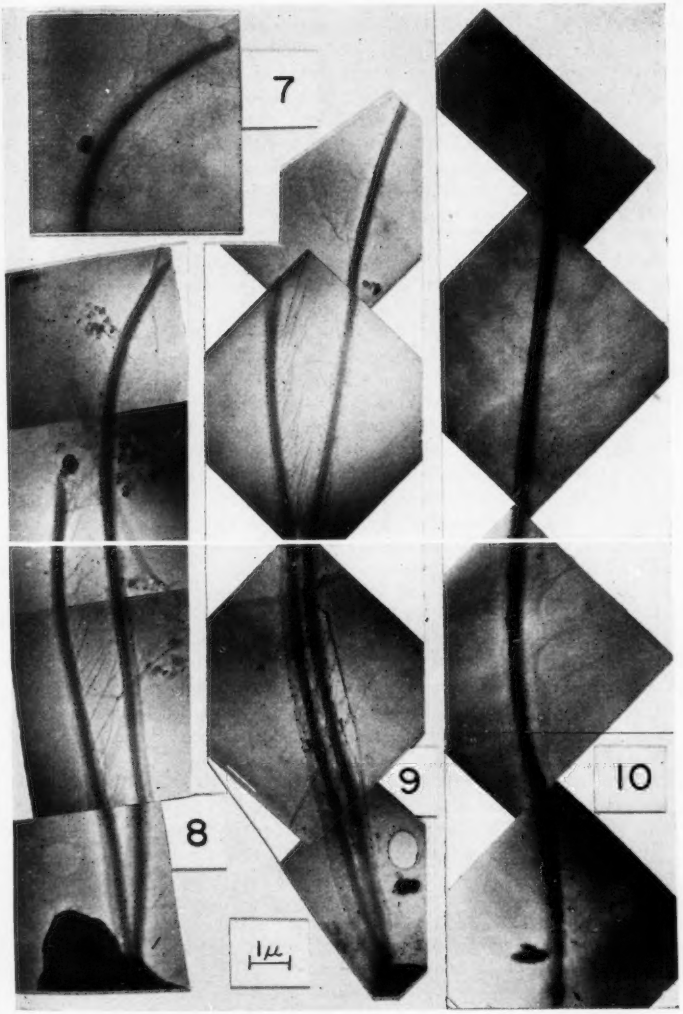
The axoneme is of approximately uniform diameter throughout the length of the flagellum. A dark axial line suggests the presence of longitudinally oriented fibrils in the axoneme.

Previous workers have depicted *Chilomonas* flagella as ending bluntly (Owen, 1949) or as ending in a lash (Awerinzew, 1907). No one, however, has heretofore demonstrated the presence of flimmer upon *Chilomonas* flagella. As may be seen in figs. 8 and 9, they are definitely present along the inner side of the shorter flagellum, and a few occur along the inner margin of the longer flagellum. These flimmer appear to be unlike those of *Ochromonas* and unlike those of euglenoids (see plates of Pitelka, 1949, or Houwink, 1951). The sturdy appearance of the flimmer in fig. 8 may be the result of several filaments adhering to one another.

In seeking to explain why previous workers have failed to detect the flimmer of *Chilomonas*, we should point out that flimmer are often lacking in preparations of species known to exhibit them. Perhaps differences in cultural conditions or the physiological state of the organisms might account for the deviations in appearance.

Astasia klebsii.—Thirty-five micrographs were made of seven specimens of *Astasia*, a euglenoid monoflagellate. As in *Ochromonas* and *Chilomonas*, the flagellum consists of a relatively dense, fibrillar axoneme, and a less dense sheath (figs. 7, 10). In fig. 10, the sheath exhibits cross-striations such as were interpreted by Brown (1945) as perhaps representing a closely-wrapped helix. Houwink's concept that they are comparable to the striations of skeletal muscle is more probably correct.

Euglenoid flagella typically exhibit a single row of flimmer along one side of the flagellum. Most of our micrographs present this appearance. How-



Figs. 7-10.—7. Distal end of flagellum of *Astasia* showing tangled flimmer on both sides, and sheath substance spread out along flimmer. Technique 1; 8. Flagella and part of cell body of *Chilomonas*. Flimmer are unilateral, as in euglenoids (see fig. 10), and show distinctly on the shorter flagellum. Fibrillar structure of axoneme apparent near tip of longer flagellum. Technique 1; 9. Flagella and part of cell body of *Chilomonas*. As in fig. 8, flimmer are unilateral and show best on the short flagellum. Technique 1; 10. Portion of flagellum of *Astasia*. The unilateral flimmer are more delicate than those of *Chilomonas* (see figs. 8, 9). Note that transverse dark areas in sheath (near middle of figure) appear to alternate on the two sides of the flagellum, as would be expected if they represent helical fibrils. Technique 1.

ever, fig. 7, like some of Pitelka's micrographs of *Rhabdomonas*, shows a flagellum with flimmer extending from both sides. This might have resulted from settling of the flagellum upon the substrate with the single flimmer row directed either upward or downward, rather than to one side.

The general appearance of sheath and flimmer in these micrographs gives the impression that the flimmer are either due to extrusion of material from a semifluid sheath or that material from the sheath adhered to the fibrils as they broke away from the surface of the sheath, or that material exuded from the sheath has flowed out along the flimmer. If the flimmer of *Astasia* and *Chilomonas* originate in the manner hypothesized for *Ochromonas*, each fibril represents one entire helical turn around the flagellum, rather than a half turn as in *Ochromonas*. In *Chilomonas*, measurement of the length of the flimmer relative to the width of the flagellum bears out the hypothesis nicely; in *Astasia*, it is impossible to be precise because of difficulty in determining the boundaries of the sheath. Furthermore, if this interpretation be correct, the coils of the helix must be much closer together than in *Ochromonas*.

Pringsheim and Hovasse (1950) suggest that the flimmer seen in the electron micrographs of *Euglena* flagella (Brown, 1945) are not the same structures as those demonstrable by the Loeffler technique. The electron micrographs of Chen (1950) are no clearer, but, since his fig. 2 shows a flagellum which had not been centrifuged (Brown's specimens had been centriufged), the flimmer are arranged along one side and are less tangled. The plates of Pitelka (1949) and of Houwink (1951) are even more convincing. As we interpret such appearances, in correlating them with those obtained by the Loeffler method, it is our opinion that the Loefffler technique results in the clumping of several fibrils (Brown's flimmer). These clumps, with mordant precipitate and stain, yield the flimmer of Loeffler et al. To this extent, we might agree with Pringsheim and Hovasse in considering the flimmer depicted in Brown's micrographs as not representing the same structures as have been observed with the light microscope. Obviously, the light microscope would be incapable of resolving the delicate fibrils observable in the electron micrographs.

Pringsheim and Hovasse question Brown's interpretation that his plates show two major fibrils or fiber bundles in euglenoid flagella, preferring to interpret them as merely ribbon-like flagella. Indeed, in their plate XVI, figs. 1, 2 strongly suggest a ribbon-like structure, without showing evidence of two major bundles of fibrils. (These two figures appear to have been retouched, but not in a way which alters the basic appearance.) Nevertheless, Brown's micrographs—especially plates 2A, 5, 9—give us the impression of an axoneme composed of two major fibril bundles. Pitelka (1949) reported the same appearance. Perhaps the ribbon-like shape of the *Euglena* flagellum, discussed by Ulehla (1911) and others, results in part from the presence of two parallel bundles of fibrils within the axoneme. This, of course, is pure speculation; perhaps Pringsheim and Hovasse are entirely correct in assuming that the appearance of a double axoneme, so to speak, is due to artifact.

Another point brought out by Pringsheim and Hovasse (1950), and mentioned earlier by Pringsheim (1948), concerns the mode of flagellar attachment in *Euglena* and *Astasia*. They point out that Hollande (1942) and others have demonstrated the presence of two flagellar "roots" in both genera, and contend that these represent two distinct flagella rather than a bifurcate root of a single flagellum. Such an interpretation certainly seems reasonable

and clears up problems of relationship within the order. It also suggests a thorough revision of phylogenetic concepts proposed by Jahn (1946) and others, and renders untenable the hypothesis advanced by Brown (1945) that the two major fibrils of the euglenoid axoneme (if there be two) might represent the fused axonemes of two "ancestral" flagella. It strengthens, in a much more effective fashion, the hypothesis of biflagellate ancestry proposed by such early workers as Hartmann and Chagas (1910).

Chen (1950) observed no distinct fiber bundles in *Euglena* flagella, but found a double set in *Trachelomonas volvocina*. He states that "the root of the flagellum, when it is squeezed out from the reservoir, is seen to consist of core only." Our micrographs show nothing of the relationship of flagellar sheath to the rest of the cell, but we suspect that the sheath should extend from the base of the flagellum, being in contact with the cell body. Perhaps the basal portion of the sheath had been destroyed in these "squeezed" preparations. Concerning the flimmer of *Euglena*, Chen states: "Although they are normally present all around the flagellum, since they are soft, they trail behind when the flagellum is beating, with the result that they are present only on the convex or trailing side." Others have expressed essentially the same idea. However, we might point out that the flimmer in Brown's micrographs occur on the concave side of the flagellum as often as on the convex side, although they are obviously quite delicate and should have been "trailed" otherwise. Although they occur upon either side of the flagellum, in any one region they are predominantly located upon one side. In places where they "shift" to the opposite side, it is apparently correlated with a twist in the flagellum. In our opinion, the evidence supports the long-standing interpretation that the flimmer of euglenoids occur primarily along one side of the flagellum ("einseitswendige"), rather than all around it as stated by Chen.

Deflandre (1950) presents an excellent criticism of the papers of Owen (1947) and Pringsheim and Hovasse (1950).

CONCLUSION

Although this study contributes several new facts and suggests a reinterpretation of others, there remains a great deal to be done before the ultrastructure of protozoan flagella will be known or understood. Among the promising techniques for future investigations are shadow-casting (Williams and Wyckoff, 1945) and freeze-drying (Drummond, 1950; Mendelow and Hamilton, 1950). The methods employed in studies of mammalian spermatozoa (Bretschneider and van Iterson, 1947; Bretschneider, 1949a and 1949b; Randall and Friedlander, 1950) should suggest ways and means for protistologists to utilize the electron microscope more effectively. In order to clarify the relationship of the flagellum to the cell body (mode of attachment and origin), electron micrographs of sectioned specimens must some day be made. The papers of Bretschneider (1949c) and of Pease and Baker (1948) present possible techniques for such a study, and that of Hillier and Gettner (1950) even describes a means of obtaining serial sections.

Perhaps the most revolutionary and promising new technique is that of Anderson (1950). Since ordinary drying, or even freeze-drying, flattens specimens due to adhesive and cohesive forces of the liquids involved, he replaces the water in specimens with carbon dioxide under high pressure. Upon raising the temperature, the liquid becomes a gas which escapes and leaves delicate

structures uncollapsed. For such specimens as protozoan flagella, this method may be ideal.

SUMMARY

Previous work on flagellar structure is briefly reviewed.

This study is based upon a total of 107 electron micrographs of 33 different specimens. Electron micrographs of the flagella of *Ochromonas variabilis* Meyer (Chrysomonadina), *Chilomonas* sp. (Cryptomonadina), and *Astasia klebsii* Lemmermann (Euglenoidina) are presented and discussed. The flagella of all of these protozoons exhibit a relatively dense, fibrillar axial core or axoneme, surrounded by a less dense sheath. Diagonal or transverse lines in the sheaths of these flagella suggest the occurrence of fibrillar elements within the sheath.

Flimmer (mastigonemes) are present on the flagella of all three species studied, and are readily demonstrable following the use of Owen's fixative (contra Owen). The flimmer on *Chilomonas* flagella, which are unilateral but sturdier than those of *Astasia*, have never before been demonstrated. The flimmer on *Ochromonas* flagella appear relatively straight and rigid, and often arise from evenly-spaced protuberances along both sides of the flagellum.

A hypothetical mechanism for the formation of flimmer is suggested, whereby flimmer are produced by fraying of helical fibrils within the sheath. Evidence for this interpretation is presented.

Promising techniques for future investigations are suggested.

REFERENCES

ANDERSON, THOMAS F. 1950—A new technique for the study of biological structures in the electron microscope. Biol. Bull. 99(2):315-316.

AWERINZEW, S. VON 1907—Beiträge zur Kenntnis der Flagellaten. Zool. Anz. 31(25):834-841.

BRETSCHNEIDER, L. H. 1949a—An electron-microscopical study of bull sperm III. Proc. Konink. Nederl. Akad. van Wetenschappen 52(3):301-309.

———1949b—An electron-microscopical study of sperm IV. (The spermtail of bull, horse and dog). Ibid. 52(5):526-534.

———1949c—A simple technique for the electron-microscopy of cell and tissue sections. Ibid. 52(6):654-666.

——— AND WOUTERA VAN ITERSON 1947—An electron-microscopical study of bull sperm I. Ibid. 50(1):88-97.

BROWN, HARLEY P. 1945—On the structure and mechanics of the protozoan flagellum. Ohio Jour. Sci. 45(6):247-301.

CHEN, Y. T. 1950—The flagellar structure of some protista. Proc. Conf. Electron Micros., Delft 1949:156-158.

COX, AVALEE 1950—A study of protozoan flagella. Master's Thesis, Univ. Okla., Norman. 39 pp.

DEFLANDRE, G. 1923—Emploi de la nigrosine dans l'étude des algues inferieures. Bull. Soc. bot. de France 70:738-741.

———1934—Sur la structure des flagelles. Ann. Protist. 4:31-54.

———1950—A propos des euglèniens et de la structure des flagelles. Arch. Zool. Exp. et Gén. 87 (Notes et Revue, No. 2):61-68.

DELLINGER, O. P. 1909—The cilium as a key to the structure of contractile protoplasm. Jour. Morph. 20:171-210.

DE ROBERTIS, E. AND F. O. SCHMITT 1948—An electron microscope analysis of certain nerve axon constituents. Jour. Cell. Comp. Physiol. 31(1):1-24.

DRUMMOND, D. G. 1950—The practice of electron microscopy. Jour. Royal Micr. Soc. Series 3, 70(1):1-141.

FISCHER, A. 1894—Über die Geisseln einiger Flagellaten. Prings. Jahrb. f. wiss. Bot. 26:187-235.

FOSTER, E., M. B. BAYLOR, N. A. MEINKOTH AND G. L. CLARK 1947—An electron

microscope study of protozoan flagella. Biol. Bull. 93(2):114-121.
GELEI, J. VON 1926—Zur Kenntnis des Wimperapparates. Zeit. f. Anat. u. Entwickl. 81:530-553.
GERLOFF, J. 1940—Beiträge zur Kenntnis der Variabilität und Systematik der Gattung *Chlamydomonas*. Arch. f. Protistenk. 94:311-502.
HALL, C. E., M. A. JAKUS AND F. O. SCHMITT 1946—An investigation of cross striations and myosin filaments in muscle. Biol. Bull. 90(1):32-50.
HARTMANN, M. AND C. CHAGAS 1910—Flagellaten-Studien. Mem. Inst. Oswaldo Cruz 2:64-125.
HILLIER, JAMES AND M. E. GETTNER 1950—Sectioning of tissue for electron microscopy. Sci. 112(2914):520-523.
HOLLANDE, ANDRÉ 1942—Étude cytologique et biologique de quelques flagellés libres. (Protistologica XCI). Arch. Zool. Exp. et Gen. 83 (Fasc. 1):1-268.
HOUWINK, A. L. 1951—An E.M. study of the flagellum of *Euglena gracilis*. Proc. Konink. Nederl. Akad. van Wetenschappen, Series C, 54(2):132-137.
HYMAN, L. H. 1940—The invertebrates: Protozoa through Ctenophora. McGraw-Hill, New York.
JAHN, THEODORE L. 1946—The euglenoid flagellates. Quart. Rev. Biol. 21(3):246-274.
KIRBY, HAROLD 1947—Methods in the study of Protozoa. Univ. Calif. Press, Berkeley.
KORSCHIKOV, A. 1923—Über den Bau und die Aggregation der Geisseln bei den Volvocales und Flagellaten. Archs. Soc. Russe de Protistol. 2:195-205.
KUNSTLER, J. 1889—Recherches sur la morphologie des flagellés. Bull. Sci. de la France et de la Belgique 20:399-515.
LOEFFLER, F. 1889—Eine neue Methode zum Färben der Mikroorganismen, im besonderen ihrer Wimperhaare und Geisseln. Centralbl. f. Bakt. 6:209-224.
MANTON, I. AND B. CLARKE 1951—Demonstration of compound cilia in a fern spermatozoid with the electron microscope. Jour. Exp. Bot. 2(1):125-128.
MENDELOW, HARVEY AND J. B. HAMILTON 1950—A new technique for rapid freezing and dehydration of tissues for histology and histochemistry. Anat. Rec. 107(4):443-451.
MILLER, GLORIA S. 1949—Calibration of the electron microscope and techniques for specimen preparation. Master's Thesis, Univ. Okla., Norman.
OWEN, H. MALCOLM 1947—Flagellar structure: I. A discussion of fixation and staining of the protozoan flagellum. Trans. Amer. Micr. Soc. 66(1):50-58.
———1949—Flagellar structure: II. The flagellum as a taxonomic character. Ibid. 68(3):261-274.
PEASE, D. C. AND R. F. BAKER 1948—Sectioning techniques for electron microscopy using a conventional microtome. Proc. Soc. Exp. Biol. and Med. 67(4):470-474.
PETERSEN, J. B. 1929—Beiträge zur Kenntnis der Flagellatengeisseln. Botanisk Tidsskrift (Copenhagen) 40(5):373-389.
PITELKA, DOROTHY R. 1949—Observations on flagellum structure in Flagellata. Univ. Calif. Publ. Zool. 53(11):377-430.
PRINGSHEIM, E. G. 1948—Taxonomic problems in the Euglenineae. Biol. Revs. 23:46-61.
——— AND R. HOVASSE 1950—Les relations de parenté entre Astasiacées et Euglènacées. Arch. Zool Exp. et Gén. 86 (Fasc. 8):499-549.
RADIO CORPORATION OF AMERICA 1946—Electron Microscope Instructions. Camden, New Jersey.
RANDALL, J. T. AND M. H. G. FRIEDLANDER 1950—The microstructure of ram spermatozoa. Exp. Cell Research 1(1):1-32.
ROZSA, G., A. SZENT-GYÖRGYI AND R. W. G. WYCKOFF 1950—The fine structure of myofibrils. Ibid. 1(2):194-205.
SCHMITT, F. O. 1944—Structural proteins of cells and tissues. Advances in Protein Chemistry 1:25-68.
———, C. E. HALL AND M. A. JAKUS 1943—The ultrastructure of protoplasmic fibrils. Biological Symposia 10:261-276.
ULEHLA, V. 1911—Ultramikroskopische Studien über Geisselbewegung. Biol. Centralbl. 31:645-731.
VLK, W. 1938—Über den Bau der Geissel. Arch. f. Protistenk. 90:448-488.
WILLIAMS, R. C. AND R. W. G. WYCKOFF 1945—Electron shadow-micrography of virus particles. Proc. Soc. Exp. Biol. and Med. 58(3):265-270.

A Catalogue of the Protozoa and Helminths of North American Rodents. I. Protozoa and Acanthocephala

David J. Doran*

Animal Disease and Parasite Research Branch; Agricultural Research Center, Beltsville, Maryland

This is the first of four papers summarizing the host and geographical distribution records of the protozoa and helminths reported from North American rodents (Order Rodentia). The most recent catalogue of this type covering all of the rodent genera is that of Harkema (1936). Because interest in the parasitic fauna of rodents has increased rapidly since that time, and since the writer is privileged to have access to the Index-Catalogue of Medical and Veterinary Zoology, Animal Disease and Parasite Research Branch, it seemed desirable to gather together once again the many widely dispersed records for the benefit of those working in the field.

This catalogue covers references through 1952 and includes only natural infections of rodents occurring in continental North America. It does not include records from hamsters, guinea pigs, or rodents in Zoological Parks that were obtained from localities outside North America. However, it does include reports concerning rodents in Zoological Parks that were obtained from localities within North America and these are indicated as such with an asterisk (*).

The common laboratory white rat and white mouse are referred to as *Rattus norvegicus* "albus" and *Mus musculus* "albus," respectively. No locality records are listed for these two hosts. Locality records concerning other hosts are listed except when the locality is not known or when merely reported as occurring in North America. Numbers in parentheses immediately following distribution records refer to bibliographical entries.

Many of the parasite names which are numbered and listed in alphabetical order are no longer recognized. There appears to be considerable difference of opinion regarding the status of others. Names with which certain of the entries have been reported as synonymous are listed in brackets followed by the authority or authorities (number in parentheses). When no authority is given, the person making a new combination is considered the authority. No new combinations have been made. However, in certain instances the writer has indicated probable synonyms not mentioned in the literature with an italicized question mark in parentheses (*?*). This is to be distinguished from a question mark lacking italics which indicates that uncertainty was expressed by another author. Host names are listed as they appear in the literature except that "ssp." has been added when the species consists of a number of subspecies and the subspecies has not been designated. Host names not commonly accepted are listed in brackets immediately following the name commonly accepted. It was thought best not to list parasites together with their respective reported synonyms because of differences of opinion regarding synonymy. Following the distribution records of certain parasite entries, reference

* This work was started at the University of California, Los Angeles.

is made to the numbers of other entries with which they have been reported as synonyms. This is done so that a more accurate idea can be gained of host and geographical distribution.

ACKNOWLEDGEMENT

It is a pleasure to acknowledge the helpful assistance and suggestions of Dr. E. W. Price, Allen McIntosh and John T. Lucker of the Animal Disease and Parasite Research Branch. Dr. David H. Johnson, Associate Curator, U. S. National Museum, provided valuable assistance with the names of rodent hosts.

Protozoa

1. *Amoeba enterica* Walker, 1908 [=*Endamoeba* (=*Entamoeba*) *muris* (Grassi, 1881) Kofoid and Swezy, 1921 (22)].—*Mus musculus*—U.S. (132); *Rattus norvegicus* [*Mus decumans*—U.S. (132)] (See Nos. 2, 3, 4, 16, 49).

2. *A. fecalis* Walker, 1908 [*Endamoeba* (=*Entamoeba*) *muris* (Grassi, 1881) (22)].—*Mus musculus*—U.S. (132); *M. m.* "albus" (132); *Rattus norvegicus* [*Mus decumans*—U.S. (132)] (See Nos. 1, 3, 4, 16, 49).

3. *A. muris* Grassi, 1881 [=*Endamoeba* (=*Entamoeba*) *muris* (Grassi, 1881) (22), and various authors].—*Mus musculus*—U.S. (132) (See Nos. 1, 2, 4, 16, 49).

4. *A. musculi* Walker, 1908 [=*Endamoeba* (=*Entamoeba*) *muris* (Grassi, 1881) (22)].—*Mus musculus*—U.S. (132) (See Nos. 1, 2, 3, 16, 49).

5. "amoeba."—*Sciurus niger rufiventer*—Kan. (56) (128).

6. *Babesia citelli* Becker and Roudabush, 1934.—*Citellus tridecemlineatus* ssp.—Iowa (16).

7. *B. wrighti* Tomlinson, Smith and Fogg, 1948.—*Citellus variegatus buckleyi*—Texas (121).

8. *B.* sp.—*Microtus p. pennsylvanicus*—Mass. (127).

9. *Balantidium coli* (Malmsten, 1857) Stein, 1862.—*Rattus norvegicus*—Md. (4), Tenn. (55).

10. *Chilomastix bettencourti* da Fonseca, 1915.—*Mus musculus*—U.S. (140); *M. m.* "albus" (119); *Peromyscus leucopus* ssp.—U.S. (140); *Rattus norvegicus*—Md. (4), N.C. (59), U.S. (140), Can. (53), Mex. (19); *R. n.* "albus" (80) (140); *Rattus rattus*—Md. (4), U.S. (140).

11. *C. instabilis* Crouch, 1926.—*Marmota monax* ssp.—Iowa, Ky. (38).

12. *C. magna* Becker, 1926.—*Citellus b. beecheyi*—Calif. (74); *C. beecheyi* ssp.—Calif. (89); *C. beldingi beldingi*—Calif. (74); *C. tridecemlineatus* ssp.—Iowa (10); *C. t. tridecemlineatus*—Mich. (74), Iowa (10).

13. *C.* sp.—*Neotoma fuscipes* ssp.—Calif. (81); *Rattus norvegicus* "albus" (81).

14. "coccidia."—*Castor* sp.—Pa. (97); *Citellus parryi* ssp.—Alas. (87); *Erethizon dorsatum* ssp—Pa* (106); *Neotoma fuscipes* ssp—Calif. (81); *Ondatra zibethica* ssp.—Md. (86); *Sciurus griseus anthonyi*—Calif. (95); *S. niger bryanti*—Md. (47); *S. n. rufiventer*—Kan. (56).

15. *Councilmania decumani* (Rudovsky, 1921) Kofoid, Swezy and Kessell, 1924 [=*Endamoeba* (=*Entamoeba*) *decumani* (Rudovsky, 1921) (146) (147)].—*Mus musculus*—Calif. (73); *Rattus norvegicus* [*Mus norvegicus*—Calif. (73)]; *R. n.* "albus" (80) (81) (See No. 45).

16. *C. muris* (Grassi, 1881) Kofoid, Swezy and Kessell, 1924 [=*Endamoeba* (=*Entamoeba*) *muris* (Grassi, 1881) Kofoid and Swezy, 1921 (146) (147)].—*Mus musculus*—Calif. (73); *M. m.* "albus" (73); *Rattus norvegicus* [*Mus norvegicus*—Calif. (73)] (See Nos. 1, 2, 3, 4, 49).

17. *Cryptosporidium muris* Tyzzer, 1910.—*Mus musculus* "albus" (125).

18. *C. parvum* Tyzzer, 1912.—*Mus musculus* "albus" (126).

19. *Eimeria beecheyi* Henry, 1932.—*Citellus beecheyi* ssp.—Calif. (68).

20. *E. bilamellata* Henry, 1932.—*Citellus lateralis* ssp. [*Callospermophilus chrysodeirus*—Calif. (68)].

21. *E. callospermophili* Henry, 1932.—*Citellus lateralis* ssp. [*Callospermophilus chrysodeirus*—Calif. (68)].

22. *E. carini* Pinto, 1928 [=*E. miyairii* Ohira, 1912 (58)].—*Rattus norvegicus*—Texas (12) (13), Can. (53) (See Nos. 32, 35).

23. *E. citelli* Kartchner and Becker, 1930.—*Citellus beecheyi* ssp.—Calif. (89); *C. tridecemlineatus* ssp.—Iowa (72), Calif. (68).

24. *E. cynomysis* Andrews, 1928.—*Cynomys* sp.—Ohio (1; animal supply house).

25. *E. dicrostonicis* Levine, 1951.—*Dicrostonyx groenlandicus richardsoni*—Alas. (87).

26. *E. dura* Crouch and Becker, 1931 [=*E. monacis* Fish, 1930 (58)].—*Marmota monax* ssp.—Iowa (39) (See No. 34).

27. *E. eubeckeri* Hall and Knipling, 1935.—*Citellus beecheyi* ssp.—Calif. (89); *C. franklinii*—Iowa (57).

28. *E. falciformis* (Eimer, 1870) Schneider, 1875.—*Mus musculus* "albus" (110) (120) (12).

29. *E. franklinii* Hall and Knipling, 1935.—*Citellus franklinii*—Iowa (57).

30. *E. geomydis* Skidmore, 1929.—*Geomys bursarius*—Nebr. (118).

31. *E. glaucomydis* Roudabush, 1937.—*Glaucomys volans* ssp.—Iowa (109).

32. *E. miyairii* Ohira, 1912 [=*E. nieschulzi* Dieben, 1924 (12)].—*Rattus norvegicus*—Md. (4), Iowa (14) (12), Texas (13), Can. (53), Mex. (19) (See Nos. 22, 35).

33. *E. mohavensis* Doran and Jahn, 1952.—*Dipodomys panamintinus mohavensis*—Calif. (45).

34. *E. monacis* Fish, 1930.—*Marmota m. monax*—D.C. (53); *M. monax* ssp.—Iowa (38) (See No. 26).

35. *E. nieschulzi* Dieben, 1924.—*Rattus norvegicus*—Md. (4), Ind. (27), N.Y. (6), Can. (53) (See Nos. 22, 32).
36. *E. neotomae* Henry, 1932.—*Neotoma fuscipes* ssp.—Calif. (68); *N. lepida monstrabilis* —Ariz. (131).
37. *E. ondatrae-zibethicae* Martin, 1930.—*Ondatra zibethica* ssp.—Nebr. (92).
38. *E. os* Crouch and Becker, 1931.—*Marmota monax* ssp.—Iowa (38).
39. *E. perforoides* Crouch and Becker, 1931.—*Marmota monax* ssp.—Iowa (38).
40. *E. residua* Henry, 1932.—*Neotoma fuscipes* ssp.—Calif. (68); *N. lepida monstrabilis*—Ariz. (131).
41. *E. separata* Becker and Hall, 1931.—*Rattus norvegicus*—Md. (4), Texas (13), Can. (53), Mex. (19) [*Epimys norvegicus*—Iowa (15)].
42. *E. sciurorum* Galli-Valerio, 1922.— *Glaucomys volans* ssp.—Iowa (109); *Sciurus carolinensis* [*Neosciurus carolinensis* (88)]; *S. niger rufiventer*—Iowa (76).
43. *E. stiedae* (Lindemann, 1865) Kisskalt and Hartmann, 1907.—*Ondatra zibethica* ssp. —Ont. (85).
44. *E.* sp.—*Ondatra zibethica* ssp.—N.Y. (25); *Sciurus g. griseus*—Calif. (68); *S. niger rufiventer*—Ohio (8), Kan. (56) (128).
45. *Endamoeba decumani* (Rudovsky, 1921).—(See No. 15).
46. *E.* (=*Entamoeba*) *dipodomysi* Hegner, 1926.—*Dipodomys a. agilis*—Calif. (43); *D. heermanni morroensis*—Calif. (43); *D. m. merriami*—Calif. (43); *D. panamintinus mohavensis* —Calif. (43); *D. spectabilis* ssp—N.M. (64).
47. *E.* (=*Entamoeba*) *histolytica* (Schaudinn, 1903) Hickson, 1909.—*Mus musculus*—Tenn. (55); *Rattus norvegicus*—Md. (4) (2), Mo. (124) [*Mus norvegicus*—S.C. (91)]; *R. n.* "albus" (32).
48. *E.* (=*Entamoeba*) *marmotae* Crouch, 1936.—*Marmota monax* ssp.—Iowa, Ky. (37).
49. *E.* (=*Entamoeba*) *muris* (Grassi, 1881) Kofoid and Swezy, 1921.—*Mus musculus*—N.C. (59), Tenn. (55); *M. m.* "albus" (120); *Rattus norvegicus*—Md. (2) (4), N.C. (59), Tenn. (55), Ont. (53), Mex. (19); *R. n.* "albus"—(114) (See Nos. 1, 2, 3, 4, 16).
50. *E.* (=*Entamoeba*) *ratti* Kessel, 1924.—*Rattus norvegicus* [*Mus norvegicus*—Calif. (73)].
51. *E.* (=*Entamoeba*) *citelli* Becker, 1926.—*Citellus beecheyi* ssp.—Calif. (89); *Citellus tridecemlineatus* ssp.—Iowa (9); *C. t. texensis*—Iowa (9).
52. *Endolimax nana* (Wenyon and O'Connor, 1917) Brug, 1918.—*Rattus norvegicus*—Md. (4); *R. n.* "albus" (32).
53. *E. ratti* Chiang, 1925.—*Rattus norvegicus*—Ind. (27).
54. *Giardia beckeri* Hegner, 1926 [=*G. duodenalis* var. *beckeri* (Hegner, 1926) Filice, 1952 (52)].—*Citellus beecheyi* ssp.—Calif. (89); *C. tridecemlineatus* ssp.—Iowa (10) (See No. 56).
55. *G. duodenalis* (Duvaine, 1875) Stiles, 1902.—See Nos. 58, 83).
56. *G. duodenalis* var. *beckeri* (Hegner, 1926) Filice, 1952.—*Citellus beecheyi* ssp.—Calif. (52) (See No. 54).
57. *G. duodenalis* var. *lamblia* (Stiles, 1915) Filice, 1952.—(See No. 63).
58. *G. duodenalis* var. *duodenalis* (Davaine, 1875) Filice, 1952.—(See Nos. 55, 83).
59. *G. duodenalis* var. *microti* (Kofoid and Christiensen, 1915) Filice, 1952.—(See No. 64).
60. *G. duodenalis* var. *perognathi* Filice, 1952.—*Dipodomys a. agilis*—Calif. (43); *D. m. merriami*—Calif. (43); *Perognathus californicus* ssp.—Calif. (52).
61. *G. doudenalis* var. *peromysci* Filice, 1952.—*Peromyscus californicus* ssp.—Calif. (52).
62. *G. duodenalis* var. *simoni* (Lavier, 1924) Filice, 1952.—*Rattus norvegicus* "albus" (52) (See No. 67).
63. *G. lamblia* Stiles, 1915 [=*G. duodenalis* var. *lamblia* (Stiles, 1895) Filice, 1952 (52)]. —*Rattus norvegicus* "albus" (65) (103) (See No. 57).
64. *G. microti* Kofoid and Christiensen, 1915 [=*G. duodenalis* var. *microti* (Kofoid and Christiensen, 1915) Filice, 1952 (52)]—*Microtus* sp.—Nova Scotia (111); *M. c. californicus*—Calif. (21) (79) (20); *M. pennsylvanicus acadius*—Nova Scotia (111); *Mus musculus*—Calif. (79); *Peromyscus maniculatus gambelii*—Calif. (79) [*P. gambelii*—Calif. (21)]; *P. l. leucopus* —N.C. (59); *Rattus norvegicus* [*Epimys norvegicus*—Calif. (79)] (See no. 59).
65. *G. muris* (Grassi, 1879) Kofoid and Christiensen, 1915.—*Microtus c. californicus*—Calif. (78); *Mus musculus*—Calif. (78); *M. m.* "albus" (120) (78); *Ondatra zibethica* ssp.—Md. (5); *Peromyscus maniculatus gambelii*—Calif. (78) [*P. gambelii*—Calif. (21)]; *Rattus rattus*—Md. (4); *R. norvegicus*—Md. (4) (62), Mo. (124), Ont. (53), Mex. (19); *R. n.* "albus" (61) (103) (80).
66. *G. ondatrae* Travis, 1939.—*Ondatra zibethica osooyoensis*—Colo. (7); *O. zibethica* ssp.—Iowa (123).
67. *G. simoni* Lavier, 1924 [=*G. duodenalis* var. *simoni* (Lavier, 1924) Filice, 1952 (52)]. —*Rattus norvegicus* "albus" (65) (See No. 62).
68. *G.* sp.—*Dipodomys heermanni* ssp.—Calif. (70); *Microtus californicus* ssp.—Calif. (52).
69. *G.* sp. (*muris* ?).—*Rattus norvegicus* "albus" (81).
70. *G.* sp. (*ondatrae* ?).—*Ondatra zibethica rivalica*—La. (102).
71. *Haemogregarina citellicola* (Wellman and Wherry, 1910) Brumpt, 1913.—(See No. 84).
72. *H.* sp.—*Microtus p. pennsylvanicus*—Mass. (127).
73. *Hepatazoon muris* (Balfour, 1905).—*Rattus norvegicus*—Md. (4), N.Y. (69), Tenn. (51), Mex. (19); *R. rattus*—U.S. (23) (See No. 74).
74. *H. perniciosum* Miller, 1908 [=*H. muris* (Balfour, 1905) (148)].—*Rattus norvegicus* —D.C. (104); *R. n.* "albus" [*Mus decumans* "albus" (94)] (See No. 73).
75. *Hexamastix muris* (Wenrich, 1924) Wenrich, 1930.—*Citellus b. beecheyi*—Calif. (74), *C. beldingi beldingi*—Calif. (74); *C. lateralis chrysodeirus*—Calif. (74); *C. t. tridecemlineatus*

—Mich. (74); *Perognathus californicus* ssp.—Calif. (74); *R. rattus*—Pa. (74), U.S. (140) (See No. 98).

76. *Hexamita marmotae* Crouch ,1934.—*Marmota monax* ssp.—Iowa (36).

77. *Hexamitus muris* (Grassi, 1881).—*Rattus norvegicus*—Md. (4), Mo. (124), Mex. (19); *R. n.* "albus" (61) (80) (81).

78. *H. pulcher* Becker, 1926.—*Citellus beecheyi* ssp.—Calif. (89); *C. b. beecheyi*—Calif. (74); *C. beldingi beldingi*—Calif. (74); *C. tridecemlineatus* ssp.—Iowa (10); *C. t. texensis*—Iowa (10); *Mus musculus*—U.S. (140); *Peromyscus leucopus* ssp.—U.S. (140); *Rattus norvegicus*—Calif. (74), U.S. (140), Mex. (19); *R. n.* "albus" (140).

79. *H. teres* Kirby and Honigberg, 1949.—*Citellus b. beecheyi*—Calif. (74); *C. beldingi beldingi*—Calif. (74); *C. t. tridecemlineatus*—Mich. (74).

80. *H.* sp.—*Citellus beecheyi* ssp.—Calif. (89); *C. tridecemlineatus* ssp.—Iowa (10); *Mus musculus* "albus" (3); *Rattus norvegicus*—Md. (2).

81. *Iodamoeba* sp.—*Rattus norvegicus*—Mex. (19).

82. *Klosiella muris* Smith and Johnson, 1902.—*Mus musculus*—U.S. (115).

83. *Lamblia duodenalis* (Davaine, 1875) Stiles, 1902 [=*Giardia duodenalis* (Davaine, 1875) Deschiens, 1921 (22); =*G. d.* var. *duodenalis* (Davaine, 1875) Filice, 1952 (52)].—*Microtus p. pennsylvanicus* [*Microtus arvalis*—U.S. (?) (117)]; *Mus musculus*—U.S. (?) (117); *Rattus norvegicus* [*Mus decumans*—U.S. (?) (117)]; *R. rattus* [*Mus rattus*—U.S. (?) (117)] (See Nos. 55, 58).

84. *Leucocytozoon citellicola* Wellman and Wherry, 1910 [=*Haemogregarina citellicola* (Wellman and Wherry, 1910) Brumpt, 1913 (23) (148)].—*Citellus b. beecheyi* [*Otospermophilus beecheyi*—Calif. (136)] (See No. 71).

85. *Monocercomoides pilleata* Kirby and Honigberg, 1949.—*Citellus b. beecheyi*—Calif. (74); *C. beldingi beldingi*—Calif. (74); *C. lateralis chrysodeirus*—Calif. (74); *C. t. tridecemlineatus*—Mich. (74) (See No. 113).

86. *M.* sp.—*Geomys bursarius*—Pa. (?) (142); *Thomomys bottae* ssp.—Pa. (?) (141).

87. *Pentatrichomastix muris* Wenrich, 1924.—"rat" (*Rattus norvegicus* ?)—U.S. (139).

88. *Pentatrichomonas* sp.—"rat" (*Rattus norvegicus* ?)—U.S. (139); *R. norvegicus*—Md. (4), U.S. (140); *R. n.* "albus" (140).

89. "pentatrichomonad."—*Microtus pennsylvanicus* ssp.—Pa. (?) (141); *Ondatra zibethica* ssp.—Pa. (?) (141); *Peromyscus leucopus* ssp.—Pa. (?) (141).

90. *Retortomonas* sp.—*Marmota monax* ssp.—U.S. (140); *Peromyscus nuttali*—U.S. (141); *Rattus norvegicus*—Md. (4); *Thomomys bottae* ssp.—U.S. (140).

91. *Sarcocystis muris* Blanchard, 1885.—*Rattus norvegicus*—Md. (104), Mo. (124).

92. *S.* sp.—*Rattus norvegicus*—Md. (4); *R. rattus*—Md. (4).

93. *Schizotrypanum cruzi* Chagas, 1909 [=*Trypanosoma cruzi* Chagas, 1909 (34)].—*Rattus norvegicus*—Mex. (18) (19) (See Nos. 119, 126).

94. *Sphaerita endamoebae* Becker, 1926.—*Citellus tridecemlineatus* ssp.—Iowa (9, in *Endamoeba citelli*).

95. *S. trichomonodis* Crouch, 1933.—*Marmota monax* ssp.—Iowa, Ky. (35, in *Trichomonas wenrichi*).

96. "sporozoa."—*Sciurus niger rufiventer*—Kan. (128).

97. *Tetramitus rostratus* Perty, 1852.—"rat" (*Rattus norvegicus* ?)—Pa. (26).

98. *Tetratrichomastix citelli* Becker, 1926 [=*Hexamastix muris* (Wenrich, 1924) (74)].—*Citellus beecheyi* ssp.—Calif. (89); *C. tridecemlineatus* ssp.—Iowa (10); *C. t. texensis*—Iowa (10) (See No. 75).

99. *Thelohania reniformis* Kudo and Hetherington, 1922.—*Mus musculus*—Ill. (84, in *Protospirura muris*).

100. *Trichomonas cryptonucleata* Crouch, 1933.—*Marmota monax* ssp.—Iowa (35).

101. *T. cynomsi* Hegner and Ratcliffe, 1927.—*Cynomys* sp.—Ohio (67).

102. *T. digranula* Crouch, 1933.—*Marmota monax* ssp.—Iowa, Ky. (35).

103. *T. intestinalis* Wenyon, 1907 [=*Tritrichomonas muris* (Grassi, 1879) Kofoid, 1920 (74)]—*Rattus norvegicus* [*Mus norvegicus*—S.C. (91)] (See Nos. 107, 108, 114).

104. *T. marmotae* Crouch, 1933.—*Marmota monax* ssp.—Iowa (35).

105. *T. microti* Wenrich and Saxe, 1950 [previously reported as *T.* (*Pentatrichomonas*) *hominis*-like flagellate by (143)].—*Microtus pennsylvanicus* ssp.—Pa. (144); *Peromyscus leucopus* ssp.—Pa. (?) (144); *Rattus norvegicus*—Pa. (?) (144).

106. *T. minuta* Wenrich, 1924 [= (?) *Tritrichomonas minuta* (Wenrich, 1924)].—"mouse" (*Mus musculus* ?)—U.S. (139); *M. musculus* (96); "rat" (*Rattus norvegicus* ?)—U.S. (139); *R. norvegicus*—Md. (4), Mex. (19); *R. n.* "albus" (145) (See No. 115).

107. *T. muris* (Grassi, 1879) [*Tritrichomonas muris* (Grassi, 1879) (77)].—*Citellus beecheyi* ssp.—Calif. (89); *C. tridecemlineatus texensis*—Iowa (10); "mouse" (*Mus musculus* ?)—*Pa.* (?) (128); *M. musculus*—N.C. (59); *M. m.* "albus" (81) (120, in *Aspicularis tetraptera*), [(119) (120) in *Syphacia obvelata*]; *Neotoma fuscipes* ssp.—Calif. (81); *Peromyscus l. leucopus*—U.S. (138); *P. maniculatus gambelii*—Calif. (78) (82); "rat" (*R. norvegicus* ?)—Pa. (?) (139), (11, in *Syphacia obvelata*); *Rattus norvegicus*—Md. (4), N.C. (59), Mo. (123), Can. (53), Mex. (19); *R. n.* "albus" (105) (61) (63) (145) (80) (81); *R. rattus*—Md. (4) (See Nos. 103, 108, 114).

108. *T. muris* var. *citelli* Becker, 1926 [=*Tritrichomonas muris* (Grassi, 1879) (74)].—*Citellus tridecemlineatus*—Iowa (10) (See Nos. 103, 107, 114).

109. *T. parva* Alexeiff, 1911 [=*T. wenyoni* Wenrich, 1946 (141); (?)=*Tritrichomonas parva* (Alexeiff)].—*Mus musculus* (96); "rat" (*R. norvegicus* ?)—*Pa.* (?) (139); *Rattus norvegicus*—Md. (4), Mex. (19); *R. n.* "albus" (66) (105) (145); *R. rattus*—Md. (4) (See Nos. 111, 116).

110. *T. wenrichi Crouch*, 1933.—*Marmota monax* ssp.—Ky. (35).

111. *T. wenyoni* Wenrich, 1946 [new name for *Trichomonas parva* Alexeiff (141)]—*Rattus norvegicus* "albus" (141) (See Nos. 109, 116).

112. *T.* sp.—*Ondatra zibethica rivalica*—La. (102); *O. zibethica* ssp. (96); *Rattus norvegicus*—Md. (2), Tenn. (55).

113. *T.* sp. of Becker, 1926 [=*Monocercomoides pilleata* Kirby and Honigberg, 1949 (74)]. —*Citellus tridecemlineatus* ssp.—Iowa (10); *C. t. texensis*—Iowa (10) (See No. 85).

114. *Tritrichomonas muris* (Grassi, 1879) Kofoid, 1920.—*Citellus lateralis bernardinus*—Calif. (74); *C. l. chrysodeirus*—Calif. (74); *Dipodomys a. agilis*—Calif. (44); *D. nitratoides brevinasus*—Calif. (44); *D. panamintinus mohavensis*—Calif. (44); *D. p. panamintinus*—Calif. (44); *D. venustus* ssp.—Calif. (74); *Mus musculus*—U.S. (141); *M. m.* "albus" (141); *Neotoma lepida* ssp.—Calif. (44); *Perognathus formosus mohavensis*—Calif. (44); *P. longimembris longimembris*—Calif. (44); *P. parvus* ssp.—Calif. (44); *Peromyscus californicus* ssp.—Calif. (44); *P. maniculata gambelii*—Calif. (44); *Rattus norvegicus*—Ind. (27), U.S. (141); *R. n.* "albus"—(141); *R. rattus*—U.S. (139) (See Nos. 103, 107, 108).

115. *T. minuta* (Wenrich, 1924).—*Mus musculus*—U.S. (140); *M. m.* "albus"—(140); *Peromyscus leucopus* ssp.—U.S. (140); *Rattus norvegicus*—U.S. (140); *R. n.* "albus"—(140) (See No. 106).

116. *T. parva* (Alexeiff, 1911) Kofoid, 1920.—*Rattus norvegicus*—U.S. (140); *R. n.* "albus" (140) (See Nos. 109, 111).

117. *Trypanosoma citelli* Watson, 1912 [=*Trypanosoma spermophili* Lavier, 1911 (148)]. —*Citellus richardsonii* ssp.—Alba. (135) (See No. 131).

118. *T. conorhini* (Donovan, 1909) Short and Swaminath, 1928.—*Rattus norvegicus*—Mex. (18).

119. *T. cruzi* Chagas, 1909.—*Hodomys alleni*—Mex. (24); *Mus musculus*—Texas (100) (99); *Neotoma a. albigula*—Ariz. (153); *N. fuscipes* ssp.—Calif. (83); *N. f. annectens*—Calif. (154); *N. f. macrotis*—Ariz., Calif. (153) (152); *N. m. micropus*—Texas (100) (99); *Peromyscus boylii rowleyi*—Calif. (153); *P. truei gilberti*—Calif. (153) (See Nos. 93, 126).

120. *T. duttoni* Thiroux, 1900.—"mouse" (*Mus musculus* ?)—(117).

121. *T. evotomys* Hadwen, 1912.—*Clethrionomys gapperi saturatus* [*Evotomys saturatus*—B.C. (135)].

122. *T. hixsoni* Becker and Roudabush, 1934.—*Citellus franklinii*—Iowa (16).

123. *T. iowensis* Becker and Roudabush, 1934.—*Citellus tridecemlineatus* ssp.—Iowa (16).

124. *T. lewisi* (Kent, 1880) Mesnil, 1901.—*Neotoma fuscipes macrotis*—Calif. (149); *N. f. annectens*—Calif. (149); *N. micropus* ssp.—Texas (48); *Rattus norvegicus*—Md. (2) (4), D.C. (104), N.Y. (69), Can. (135), N.C. (59), Mex. (17) (19), Ind. (27), Tenn. (51); *R. rattus* —Md. (4); "rat" (*R. norvegicus* ?)—Miss. (134).

125. *T. microti* Laveran and Petit, 1909.—*Microtus californicus* ssp.—Calif. (149); *M. c. sanctigiegi*—Calif. (154) (153); *M. pennsylvanicus* ssp. (33).

126. *T. neotomae* Kofoid and McCollough, 1916 [=*T. cruzi* Chagas (148)].—*Neotoma a. albigula*—Ariz. (153); *N. a. melanura*—Mex. (153); *N. fuscipes annectens*—Calif. (153) (154) (150); *N. f. macrotis*—Calif. (150) (154) (153); *N. l. lepida*—Calif. (153) (154) (See Nos. 93, 119).

127. *T. otospermophili* Wellman and Wherry, 1910.—*Citellus beecheyi* ssp.—Calif. (71) [*Otospermophilus beecheyi*—Calif. (136)].

128. *T. parkeri* Dias, 1938 [reported as *Trypanosoma* sp. by (41)].—*Marmota flaviventris nosophora*—Mont. (42).

129. *Peromysci* Watson, 1912.—*Peromyscus boylii rowleyi*—Ariz., Calif. (153) (152); *P. californicus insignis*—Calif. (152) (153); *P. maniculatus blandus*—Ariz. (153); *P. maniculatus* ssp.—Alba. (135); *P. m. gambelii*—Calif. (152) (153); *P. m. nebrascensis* [*Peromyscus nebrascensis*—Alba. (135)]; *P. truei gilberti*—Calif. (153); *P. t. truei*—Ariz. (153).

130. *T. sigmodoni* Culbertson, 1941.—*Sigmodon hispidus littoralis*.—Fla. (39).

131. *T. spermophili* Lavier, 1911.—(See No. 117).

132. *T. zapi* Davis, 1952—*Zapus princeps alleni*—Calif. (40).

133. *T.* sp.—*Citellus* sp.—Can. (135); *Microtus p. pennsylvanicus*—Mass. (127); *Neotoma a. albigula*—Ariz. (151); *Peromyscus t. truei*—Calif. (151); *Sigmodon hispidus* ssp.—Texas (48).

134. *T.* sp. (*lewisi*-like).—*Microtus californicus* ssp.—Calif. (40); *Peromyscus maniculatus* ssp.—Calif. (40); *P. boylii* ssp.—Calif. (40); *P. truei* ssp.—Calif. (40); *Reithrodontomys megalotis* ssp.—Calif. (40); *Zapus trinotatus*—Calif. (40).

Acanthocephala

1. *Echinorhynchus moniliformis* Bremser, 1811 [=(?) *M. moniliformis* (Bremser, 1811) Travassos, 1915 (122)].—*Sciurus niger* ssp. [*S. vulpinus* (31)] (See Nos. 3, 8).

2. *E. moniliformis* Bremser, 1811 of Stiles and Hassall, 1894 (Leidy collection) [=*Moniliformis clarkii* (Ward, 1917) (129)].—*Sciurus niger* ssp.—Md. (118) (See Nos. 4, 6, 7, 9, 10, 12).

3. *Gigantorhynchus moniliformis* (Bremser, 1811) Railliet, 1893 [=*Moniliformis moniliformis* (Bremser, 1811) (122)].—*Rattus norvegicus* [*Mus decumans* (117)]; *R. n.* "albus" [*Mus norvegicus* "albus" (117)] (See Nos. 1, 8).

4. *Hormorhynchus clarkii* Ward, 1917 [=*Moniliformis clarkii* (Ward, 1917) (129) (93)]. —"squirrel"—Ill. (132) (See Nos. 2, 6, 7, 9, 10, 12).

5. *Macracanthorhynchus hirudinaceus* (Pallas, 1781) Travassos, 1917.—*Sciurus niger* ssp. —Ohio (60); *S. n. rufiventer*—Ohio (107) (108); *Tamias striatus* ssp.—Ohio (107).

6. *Moniliformis dubius* Meyer, 1932.—(See Nos. 2, 4, 7, 9, 10, 12).

7. *M. clarkii* (Ward, 1917) Van Cleave, 1924.—*Citellus tridecemlineatus* ssp.—Okla. (129); *Glaucomys volans querceti*—Fla. (30); *Onychomys torridus* ssp.—Calif. (46); *Sciurus* sp.—Okla. (129); *S. c. carolinensis*—Fla. (30); *S. niger* ssp.—Ill. (129), Ohio (60); *S. n. niger* —Fla. (30) (See Nos. 2, 4, 6, 9, 10, 12).

8. *M. moniliformis* (Bremser, 1911) Travassos, 1915.—*Rattus norvegicus*—D.C. (104), Md. (90); *R. rattus*—Texas (28) (See Nos. 1, 3).

9. *M. moniliformis* (Bremser) of Chandler, 1921 [=(?)*M. dubius Meyer*, 1932 (93)].— *Rattus norvegicus*—Texas (28) (See Nos. 2, 4, 6, 7, 10, 12).

10. *M. spiradentatis* McLeod, 1933 [=*M. dubius* Meyer (130); =*M. clarkii* (Ward, 1917) (30)].—*Citellus tridecemlineatus* ssp.—Man. (98) (See Nos. 2, 4, 6, 7, 9, 12).
11. *M.* sp.—*Eutamias minimus jacksoni*—Minn. (108); *Neotoma* sp.—Fla. (116); *Peromyscus maniculatus gracilis*—Minn. (50) (49); *Sciurus niger rufiventer*—Okla. (101).
12. *M.* sp. of Chandler, 1921 [=*M. dubius* Meyer (93) (29)].—*Rattus norvegicus* [*Epimys norvegicus*—Texas (28)]; *R. rattus* [*Epimys alexandrinus*—Texas (28); *Epimys rattus*—Texas (28)]; *Sciurus niger* ssp.—Texas (28) (See Nos. 2, 4, 6, 7, 9, 10).
13. *Polymorphus* sp.—*Ondatra zibethica* ssp.—B.C. (75).

Note: A host-index to the parasite names listed herein will be found at the end of Part II (Cestoda), which will appear in the next issue of this journal.

REFERENCES

1. ANDREWS, J. M. 1928—New species of coccidia from the skunk and prairie dog. J. Parasit. 14:193-194.
2. ———1934—*Endamoeba histolytica* and other protozoa in wild rats caught in Baltimore. Ibid. 20:334.
3. ——— AND M. REYNOLDS 1941—*Hexamita* in the blood of a mouse. Ibid. 27(6):26.
4. ——— AND M. WHITE 1936—An epidemiological study of protozoa parasitic in wild rats in Baltimore with special reference to *Endamoeba histolytica*. Amer. J. Hyg. 24(1):194-206.
5. ARMAGHAN, V. 1937—Biological studies on the *Giardia* of rats. Ibid. 26:236-258.
6. BAKER, D. W. 1934—Report of the diagnostic laboratory. Rep. N.Y. State Vet. Coll. (1932-1933):41-49.
7. BALL, L. 1952—Notes on helminth parasites of muskrats from western Colorado. J. Parasit. 36(5):83-84.
8. BAUMGARTNER, L. L. 1940—The fox squirrel; its life history, habits and management. Abstract, Doctoral thesis, Ohio State University.
9. BECKER, E. R. 1926—*Endamoeba citelli* sp. nov. from the striped ground squirrel, *Citellus tridecemlineatus*, and the life history of its parasite, *Sphaerita endamoebae* sp. nov. Biol. Bull. 50:444-454.
10. ———1926—The flagellate fauna of the caecum of the striped ground squirrel, *Citellus tridecemlineatus*, with special reference to *Chilomastix magna* sp. nov. Ibid. 51:287-298.
11. ———1933—Two observations on helminths. Trans. Amer. Micr. Soc. 52:361.
12. ———1934—Coccidia and coccidiosis of domesticated game and laboratory animals and of man. Collegiate Press, Inc., Ames, Iowa.
13. ——— AND R. D. BURROUGHS 1933—Rediscovery of *Eimeria carinii* Pinto, 1923. J. Parasit. 20(2):123.
14. ——— AND P. R. HALL 1931—Two species of coccidia from the Norway rat. Ibid. 18(2):115.
15. ——— AND ———1931—*Eimeria separata*, a new species of coccidia from the Norway rat (*Epemys norvegicus*). Iowa State Coll. J. Sci. 6:131-132.
16. ——— AND R. L. ROUDABUSH 1934—*Trypanosoma iowaensis* n. sp. and *Babesia citelli* n. sp. from *Citellus tridecemlineatus*, and *Trypanosoma hixsoni* n. sp. from *Citellus franklinii*. Ibid. 8:527-531.
17. BELTRÁN, E. AND P. PÉREZ 1947—Incidencia de *Trypanosoma lewisi* en ratos de la ciudad de Mexico. Rev. Inst. de Salub. Enfer. Tropic. 7(3):179-184.
18. ——— AND ———1949—Hallazgo de *Schizotrypanum cruzi* en una rata (*Rattus norvegicus*) en la ciudad de Mexico. Ibid. 10(3):235-238.
19. ——— AND ———1950—Protozoarias párasitos de las ratas en la ciudad de Mexico. Ibid. 11(1):71-78.
20. BOECK, W. C. 1917—Mitosis in *Giardia microti*. U. Calif. Publ. Zool. 18(1):1-26.
21. ———1919—Development of *Giardia microti* within the cyst. Ibid. 19:108-120.
22. ——— AND C. W. STILES 1923—Studies on various intestinal parasites (especially amoebae) of man. U.S. Pub. Health Service, Hyg. Lab. Bull. 133.
23. BRUMPT, E. 1913—Précis de Parasitologie, Paris, 2nd ed.
24. ———, L. MAZZOTI AND L. CH. BRUMPT 1939—Enquetes épidémiologieques sur la maladie de Chagas an Mexique. Ann. Parasit. Hum. et Comp. 17(4):299-312.
25. BUMP, G. 1942—31st Annual Report N.Y. State Conservation Dept.

26. BUNTING, M. 1926—Studies on the life cycle of *Tetramitus rostratus* Perty. J. Morph. 42:26-82.
27. CABLE, R. M. AND W. H. HEADLEE 1937—The incidence of animal parasites in the brown rat (*R. norvegicus*) in Tippecanoe County, Indiana. Proc. Ind. Acad. Sci. 46:217-219.
28. CHANDLER, A. C. 1921—Notes on the occurrence of *Moniliformis* sp. in rats in Texas. J. Parasit. 7:179-183.
29. ———1941—Helminths of muskrats in southeast Texas. Ibid. 27(2):174-181.
30. ———1947—Notes on *Moniliformis clarkii* in North American squirrels. Ibid. 33(3):278-281.
31. CHAPMAN, H. C. 1874—*Echinorhynchus moniliformis*. Proc. Acad. Nat. Sci. Phila. Sept., p. 76.
32. CHIANG, S. F. 1925—The rat as a possible carrier of dysentery amoeba. Ibid. 11:239-246.
33. COATNEY, G. R. 1935—A note on *Trypanosoma microti*. J. Parasit. 21:455-456.
34. CRAIG, C. F. AND E. C. FAUST 1940—Clinical Parasitology. (2nd edition) Lea and Febiger, Philadelphia.
35. CROUCH, H. B. 1933—Four new species of *Trichomonas* from the woodchuck (*Marmota monax* Linn.). J. Parasit. 19:293-301.
36. ———1934—Observations on *Hexamita marmotae* n. sp., a protozoan flagellate from the woodchuck *Marmota monax* (Linn.). Iowa State Coll. Jour. Sci. 8:513-516.
37. ———1936—The animal parasites of the woodchuck (*Marmota monax* Linn.) with special reference to protozoa. Ibid. 11:48-50.
38. ——— AND E. R. BECKER 1931—Three new species of coccidia from the woodchuck, *Marmota monax*. Ibid. 5:127-131.
39. CULBERTSON, J. T. 1941—Trypanosomiasis in the Florida cotton rat, *Sigmodon hispidus littoralis*. J. Parasit. 27(1):45-52.
40. DAVIS, B. S. 1952—Studies on the trypanosomes of some California mammals. U. Calif. Publ. Zool. 57(3):145-250.
41. DIAS, E. 1937—Trypanosomes in bat and marmot. Trans. Roy. Soc. Trop. Med. and Hyg. 31(2):260.
42. ———1938—*Trypanosoma* de um reodor, *Marmota flaviventris nosophora* achado em Montana, U.S. Livro. Jub. Travassos, pp. 141-143.
43. DORAN, D. J. 1953—The occurrence of *Tritrichomonas muris* (Grassi) in heteromyid and cricetid rodents of California. J. Parasit. 39:569.
44. ———1953—A note on *Giardia duodenalis* var. *perognathi* Filice and *Endamoeba* (=*Entamoeba*) *dipodomysi* Hegner in California rodents. *Ibid.* 39:668.
45. ——— AND T. L. JAHN 1952—Preliminary studies on *Eimeria mohavensis* sp. nov., from the kangaroo rat, *Dipodomys panamintinus mohavensis* (Grinnell). Trans. Amer. Micr. Soc. 71(2):93-101.
46. ——— AND C. P. READ 1951—A note on *Moniliformis* in southern California rodents. J. Parasit. 37(3):330.
47. DOZIER, H. L. AND H. E. HALL 1944—Observations on the Bryant flying squirrel, *Sciurus niger bryanti* (Bailey). Maryland Conservationalist 21(1):2-6.
48. EADS, R. B. AND B. G. HIGHTOWER 1952—Blood parasites of southwest Texas rodents. J. Parasit. 38(1):89-90.
49. ERICKSON, A. B. 1938—Parasites of some Minnesota rodents. J. Mammal. 19(2):252-253.
50. ———1938—Parasites of some Minnesota cricetidae and zapodidae, and a host catalogue of helminth parasites of native American mice. Amer. Midl. Nat. 20(3):575-589.
51. EYLES, D. E. 1951—Incidence of *Trypanosoma lewisi* and *Hepatozoon muris* in the Norway rat. J. Parasit. 37(3):222-224.
52. FILICE, F. P. 1952—Studies on the cytology and life history of a *Giardia* from the laboratory rat. U. Calif. Publ. Zool. 57(2):53-146.
53. FIRLOTTE, W. R. 1948—A survey of the parasites of the brown Norway rat. Can. J. Comp. Med. pp. 187-191.
54. FISH, F. 1930—Coccidia of rodents; *Eimeria monacis* n. sp. from the woodchuck. J. Parasit. 17:98-100.
55. FRYE, W. W. AND H. E. MELENEY 1932—Investigations of *Endamoeba histolyti-*

ca and other intestinal protozoa in Tennessee. II. A study of flies, rats, mice and some domestic animals as possible carriers of intestinal protozoa of man in a rural community. Amer. J. Hyg. 16:729-749.

56. GRAHAM, E. AND J. UHLRICH 1943—Animal parasites of the fox squirrel, *Sciurus niger rufiventer*, in southeastern Kansas. J. Parasit. 29(2):160-161.
57. HALL, P. R. AND E. F. KNIPLING 1935—*Eimeria franklinii* and *Eimeria eubeckeri* —2 new species of Coccidia from the Franklin ground squirrel, *Citellus franklinii*. Ibid. 2:128-129.
58. HARDCASTLE, A. B. 1943—A check list and host-index of the species of the protozoan genus *Eimeria*. Proc. Helm. Soc. Wash. 10(2):35-69.
59. HARKEMA, R. 1936—The parasites of some North Carolina rodents. Ecol. Monogr. 6(2):153-232.
60. HARWOOD, P. D. AND V. COOKE 1949—The helminths from a heavily parasitized fox squirrel, *Sciurus niger*. Ohio J. Sci. 69(4):146-148.
61. HEGNER, R. W. 1923—The effect of changes in diet on the incidence, distribution and numbers of certain intestinal protozoa of rats. Amer. J. Hyg. 3:180-200.
62. ———1923—Giardias from wild rats and mice and *Giardia caviae* sp. nov., from the guinea pig. Ibid. 3:345-353.
63. ———1924—Infection experiments with *Trichomonas*. Ibid. 4:143-151.
64. ———1926—*Giardia beckeri* n. sp. from the ground squirrel and *Endamoeba dipodomysi* from the kangaroo rat. J. Parasit. 12:203-206.
65. ———1927—Excystation and infection in the rat with *Giardia lamblia* from man. Amer. J. Hyg. 7(4):433-447.
66. ——— AND L. ESKRIDGE 1935—Susceptibility and resistance of rats to infections with trichomonad flagellates from rat and man. Ibid. 22:307-321.
67. ——— AND H. L. RATCLIFFE 1927—Trichomonads from the vagina of the monkey, mouth of cat and man, intestine of the monkey, opossum and prairie dog. J. Parasit. 14(1):27-35.
68. HENRY, D. P. 1932—Observations on coccidia of small mammals in California with descriptions of seven new species. U. Calif. Publ. Zool. 37:279-290.
69. HERMAN, C. M. 1939—A parasitological survey of wild rats in New York Zoological Park. Zoologica 24(3):305-308.
70. ———1943—*Giardia* in the blood of the kangaroo rat. J. Parasit. 29:423.
71. HOLDENREID, R., F. C. EVANS AND D. S. LONGNECKER 1951—Host-parasite-disease relationships in a mammalian community in the central coast range of California. Ecol. Monogr. 21:1-18.
72. KARTCHNER, J. A. AND E. R. BECKER 1930—Observations on *Eimeria citelli* a new species of coccidium from the striped ground squirrel. J. Parasit. 17:90-94.
73. KESSELL, J. F. 1924—The distinguishing characteristics of parasitic amoeba of culture rats and mice. U. Calif. Publ. Zool. 20(23):489-515.
74. KIRBY, H. AND B. HONIGBERG 1949—Flagellates of the caecum of ground squirrels. Ibid. 53(9):315-366.
75. KNIGHT, I. M. 1951—Diseases and parasites of the muskrat (*Ondatra zibethica*) in British Columbia. Can. J. Zool. 29(3):184-215.
76. KNIPLING, E. F. AND E. R. BECKER 1935—A coccidium from the fox squirrel, *Sciurus niger rufiventer*. J. Parasit. 21:417-418.
77. KOFOID, C. A. 1920—A critical review of the nomenclature of human intestinal flagellates, *Cercomonas, Chilomastix, Trichomonas, Tetratrichomonas*, and *Giardia*. U. Calif. Publ. Zool. 20(6):145-168.
78. ——— AND E. B. CHRISTIENSEN 1915—On binary and multiple fission in *Giardia muris* (Grassi). Ibid. 16(3):30-55.
79. ——— AND ———1915—On *Giardia microti* sp. nov. from the meadow mouse. Ibid. 16(2):23-29.
80. ———, E. MCNEIL AND A. BONESTELL 1933—Correlation of the distribution of the protozoa in the intestine of *Rattus norvegicus* with the pH concentration of the intestinal contents and wall. Ibid. 39(18):179-190.
81. ———, ——— AND ———1935—A comparison of the distribution of the intestinal protozoa of the Norway rat, wood rat, and guinea pig with reference to the hydrogen ion concentrations as determined by the glass electrode. Ibid. 41(1):1-8.

82. ——— AND O. SWEZY 1915—Mitosis and multiple fission in trichomonad flagellates. Proc. Amer. Acad. Arts and Sci. 51(6):289-378.
83. ——— AND F. D. WOOD 1933—The experimental transfer of *Typanosoma cruzi* from naturally infected *Triatoma protracta* to mammals in California. Bull. Soc. Path. Exot. 26(2):257-259.
84. KUDO, R. R. AND D. C. HETHERINGTON 1922—Notes on a microsporidian parasite of a nematode. J. Parasit. 8(3):130-133.
85. LAW, R. G. AND A. H. KENNEDY 1932—Parasites of fur-bearing animals. Bull. 4, Ontario Dept. Game and Fish.
86. LE' COMPTE, E. L. 1933—Scientific investigation of the muskrat industry in Maryland. Maryland Conservationalist 10(1):1-3.
87. LEVINE, N. D. 1951—*Eimeria dicrostonicis* n. sp., a protozoan parasite of the lemming, and other parasites from Arctic rodents. Trans. Ill. State Acad. Sci. 44:205-208.
88. ——— AND E. R. BECKER 1933—A catalogue and host-index of the species of the coccidian genus *Eimeria*. Iowa State Coll. J. Sci. 8(1):83-106.
89. LINSDALE, J. M. 1946—The California ground squirrel. A record of observations made on the Hastings Natural History Reservation. U. of Calif. Press.
90. LUTTERMOSER, G. W. 1936—A helminthological survey of Baltimore house rats (*R. norvegicus*). Amer. J. Hyg. 24:350-360.
91. LYNCH, K. M. 1915—The rat, a carrier of a dysenteric amoeba. J. Amer. Med. Assoc. 65:2232-2234.
92. MARTIN, H. M. 1930—A species of *Eimeria* from the muskrat, *Ondatra zibethica* (Linn.). Archiv Protistenkde. 70(2):273-278.
93. MEYER, A. 1932—Acanthocephala. Brauns Klassnung n. Ordnung. Tier-Reichs 4, Abt. 2, Buch 2, Lief 1:1-332.
94. MILLER, W. W. 1908—*Hepatazoon perniciosum* n. g., n. sp., a hemogregarine pathogenic for white rats, with a description of the sexual cycle in the intermediate host, a mite (*Lelaps echidninus*). U.S. Hygiene Lab. Bull. 46:1-51.
95. MOFFITT, J. 1931—Diseases reducing tree squirrel population in Southern California. Calif. Fish and Game 17(3):338-339.
96. MORGAN, B. B. 1943—Host list of the genus *Trichomonas* (Protozoa : Flagellata) Part II. Host-parasite list. Trans. Wisc. Acad. Sci. Arts and Letters 35:235-245.
97. MORLEY, L. C. 1934—Report on internal parasites of beaver. Penn. Game News 5(7):16.
98. MCLEOD, J. A. 1933—A parasitological survey of the genus *Citellus* in Manitoba. Can. J. Res. 9:108-127.
99. PACKCHANIAN, A. 1941—Reservoir hosts of Chagas' disease in the state of Texas. J. Parasit. 27(6):30.
100. ———1942—Reservoir hosts of Chagas' disease in the state of Texas. Amer. J. Trop. Med. 22:623-631.
101. PEERY, H. J. 1941—A case of peritoneal moniliformiasis in a fox squirrel. Trans. Amer. Micr. Soc. 60:375-377.
102. PENN, G. H. 1942—Parasitological survey of Louisiana muskrats. J. Parasit. 28(4):348-349.
103. POTTER, L. A. 1928—Two species of *Giardia* from the rat. Amer. J. Hyg. 8:77-84.
104. PRICE, E. W. AND B. G. CHITWOOD 1931—Incidence of internal parasites in wild rats in Washington, D.C. J. Parasit. 18(1):55.
105. RATCLIFFE, H. L. 1929—The comparative adaptability of *Trichomonas muris* and *Trichomonas parva* to culture media and changes in the intestinal flora of rats. Amer. J. Hyg. 10:63-77.
106. ———1930—Animal parasites. Rep. Lab. and Mus. Comp. Path. Zool. Soc. Phila. (1929-1930):57-59.
107. RAUSCH, R. 1946—New records of *Macracanthorhynchus hirudinaceus* in Sciuridae. J. Parasit. 32(1):94.
108. ——— AND J. D. TINER 1948—Studies on the parasitic helminths of the North Central States. I. Helminths of Sciuridae. Amer. Midl. Nat. 39(3):728-747.

109. ROUDABUSH, R. 1937—Two *Eimeria* from the flying squirrel, *Glaucomys volans*. J. Parasit. 23(1):107-108.
110. SCHWARTZ, B. 1924—First report of *Eimeria falciformis* in the United States. Ibid. 11(2):109.
111. SIMON, C. E. 1921—*Giardia* in field mice in Nova Scotia. Ibid. 7(4):190.
112. SKIDMORE, L. V. 1929—Notes on a new species of coccidia from the pocket gopher (*Geomys bursarius* Shaw). Ibid. 15:183-184.
113. SMITH, A. J. 1908—Synopsis of studies in metazoan parasitology in McManus laboratory of pathology, University of Pennsylvania. U. Penn. Med. Bull. 20(12):267-282.
114. SMITH, S. C. 1928—Host-parasite relations between *Iodamoeba williamsi* and certain mammalian hosts (guinea pigs and rats). Amer. J. Hyg. 8(1):1-15.
115. SMITH, T. AND H. P. JOHNSON 1902—On a coccidium (*Klossiella muris* gen. et spec. nov.) parasitic in the renal epithelium of the mouse. J. Exper. Med. 6(3):303-316.
116. STILES, C. W. 1932—Helminthological notes. J. Parasit. 19:90.
117. ——— AND C. G. CRANE 1910—The internal parasites of rats and mice and their relation to diseases of man. Public Health Bull., U.S. Pub. Health and Mar. Hosp. Serv., Wash.:87-110.
118. ——— AND A. HASSALL 1894—A preliminary catalogue of the parasites contained in the collection of the U.S. Bureau of Animal Industry, U.S. Army Medical Museum, Biology Department of the University of Pennsylvania (Collection of Leidy) and in the collection of Stiles and collection of Hassall. Vet. Mag.:245-253; 331-354.
119. THEILER, H. AND S. M. FARBER 1932—*Trichomonas muris*, parasitic in Oxyurids of the white mouse. J. Parasit. 19(2):169.
120. ——— AND ———1936—*Trichomonas muris*, parasitic in the oxymurid nematodes, *Aspiculuris tetraptera* and *Syphacia obvelata*, from white mice. Parasitology 28(2):149-160.
121. TOMLINSON, T. H., C. S. SMITH AND V. H. FOGG 1948—A piroplasm, *Babesia wrighti* n. sp., from the rock squirrel (*Citellus variegatus buckleyi*). J. Nat. Malaria Soc. 7(4):254-258.
122. TRAVASSOS, L. 1915—Revisão dos acantocephalos brazileiros. I. fam. Gigantorhynchidae Hamann, 1892. Brazil-Med. 29(12):89.
123. TRAVIS, B. F. 1939—Descriptions of five new species of flagellate protozoa of the genus *Giardia*. J. Parasit. 25(1):11-17.
124. TSUCHIYA, H. AND L. E. RECTOR 1935—Studies in intestinal parasites among rats caught in St. Louis. Ibid. 21(6):426-427.
125. TYZZER, E. E. 1910—An extracellular coccidium, *Cryptosporidium muris* (gen. et sp. nov.), of the gastric glands of the common mouse. J. Med. Res. 23:487.
126. ———1912—*Cryptosporidium parvum* (sp. nov.), a coccidium found in the small intestine of the common mouse. Archiv Protistenkde. 26:394-412.
127. ——— AND WEINMAN 1939—*Haemobartonella* n. g. (*Bartonella olim* pro parte), *H. microti* n. sp. of the field vole, *Microtus pennsylvanicus*. Amer. J. Hyg. 30(3):141-157.
128. UHLRICH, J. AND E. GRAHAM 1941—The animal parasites of the fox squirrel, *Sciurus niger rufiventer* and the gray squirrel, *Sciurus carolinensis carolinensis*. Anat. Rec. 81(4):65.
129. VAN CLEAVE, H. J. 1924—A critical study of the Acanthocephala described and identified by Joseph Leidy. Proc. Acad. Nat. Sci. Phila. 76:279-334.
130. ———1946—Remarques sur le genre *Moniliformis* (Acanthocèphales) et particulierement sur les espèces parasites des rats. Ann. Parasit. 21(3-4):142-147.
131. VORHIES, C. T. AND W. P. TAYLOR 1940—Life history and ecology of the white-throated wood rat, *Neotoma albigula albigula* Hartley. Tech. Bull. (86), Ariz. Agric. Exper. Station:455-529.
132. WALKER, E. L. 1908—The parasitic amoebae of the intestinal tract of man and other animals. J. Med. Res. (103), 17(4):379-459.
133. WARD, H. B. 1917—*Echinorhynchus moniliformis* in North America. J. Parasit. 3(3):141.

134. WARD, J. W. 1946—A preliminary study of the occurrence of internal parasites of animals in Mississippi. Proc. Helm. Soc. Wash. 13(1):12-14.
135. WATSON, E. A. AND S. HADWEN 1912—Trypanosomes found in Canadian mammals. Parasitology 5:11-21.
136. WELLMAN, F. C. AND W. B. WHERRY 1910—Some new internal parasites of the California ground squirrel (*Otospermophilus beecheyi*). Ibid. 3(4):417-422.
137. WENRICH, D. H. 1921—The structure and division of *Trichomonas muris* (Hartmann). J. Morph. 36(1):118-155.
138. ———1921—Specimens of protozoa from the intestines of rats and mice: their relation to those in man. Proc. Path. Soc. Phila. 23:41.
139. ———1924—The trichomonad flagellates in the caecum of rats and mice. Anat. Rec. 29(2):118.
140. ———1930—Intestinal flagellates of rats. *In* Hegner, R. and J. Andrews: Problems and methods of research in protozoology. Macmillan Co., New York:124-142.
141. ———1946—Culture experiments on intestinal flagellates. I. Trichomonad and other flagellates obtained from man and certain rodents. J. Parasit. 32(1):40-53.
142. ———1947—Culture experiments on intestinal flagellates. II. Additional observations on flagellates from man, rodents and insect larvae. Ibid. 33(1):25-28.
143. ——— AND L. H. SAXE 1948—A *Trichomonas hominis*-like flagellate with four anterior flagella from certain rodents. Anat. Rec. 101(4):658.
144. ——— AND ———1950—*Trichomonas microti* n. sp. (Protozoa; Mastigophora). J. Parasit. 36:261-267.
145. ——— AND Y. YANOFF 1927—Results of feeding active trichomonad flagellates to rats. Amer. J. Hyg. 7:119-124.
146. WENYON, C. M. 1922—On three supposed new Entamoebae of man: a review. Trop. Dis. Bull. 19(1):182.
147. ———1925—The genera *Councilmania, Karyamoeba* and *Caudamoeba.* Ibid. 20(1):333.
148. ———1926—Protozoology, New York, 2 vols.
149. WOOD, F. D. 1934—Natural and experimental infection of *Triatoma protracta* Uhler and mammals in California with American human trypanosomiasis. Amer. J. Trop. Med. 14(6):497-517.
150. ———1936—*Trypanosoma neotomae* sp. nov., in the dusky footed wood rat and the wood rat flea. U. Calif. Publ. Zool. 41(11):133-142.
151. WOOD, S. F. 1941—Chagas' disease: does it exist in man in Arizona? Southwest Med. 25(4):112-114.
152. ———1942—Observations on vectors of Chagas' disease in the United States. I. California. Bull. So. Calif. Acad. Sci. 41(2):61-69.
153. ———1952—Mammal blood-parasite records from southwestern United States and Mexico. J. Parasit. 38(1):85-86.
154. WOOD, F. D. AND S. F. WOOD 1937—Occurrence of Haematozoa in some California birds and mammals. Ibid. 23(2):197-201.
155. ——— AND ———1938—On the distribution of *Trypanosoma cruzi* Chagas in the southwestern United States. Amer. J. Trop. Med. 18(2):207-212.

Studies on Monogenetic Trematodes. XVI.
Rhamnocercinae, a New Subfamily of Dactylogyridae

L. H. Monaco, R. A. Wood and J. D. Mizelle
University of Notre Dame, Notre Dame, Indiana

Examination of the gills of *Umbrina roncador* Jordan and Gilbert, collected in the Pacific Ocean off the pier of the Scripps Institution of Oceanography, revealed the presence of three species of monogenetic trematodes. One of these represents a new subfamily, new genus, and new species, to which the present paper is devoted. The authors are grateful to Dr. Carl L. Hubbs of the above institution for generously supplying the host material, to Dr. E. W. Price and Mr. A. McIntosh of the Animal Disease and Parasite Branch (U.S.D.A.), Beltsville, Md. for checking the availability of the name *Rhamnocercus*, and to Mr. R. W. Lawrence for photographic work.

Rhamnocercinae n. subfam.

Dactylogyridae; haptor possessing conspicuous spines arranged in discrete groups, not as squamodiscs; two pairs of anchors (dorsal and ventral) present with bases connected by supporting bars; haptoral hooks present. Peduncle embellished with rows of spine-like hooks. Eyes present. Testis and ovary with entire margins, ovary pretesticular.

The closest relatives of the Rhamnocercinae are members of the subfamily Diplectaninae (Sproston 1946). Resemblances between the two subfamilies consist of the presence of 1) cuticular spines on the body proper, 2) dorsal and ventral pairs of anchors, 3) similar haptoral hooks, and 4) supporting bars associated with the anchors. Conspicuous differences are 1) the absence of squamodiscs or cuticular plates on the haptor, 2) the presence of discrete groups or clusters of spines which arise in the *parenchyma* of the haptor, and whose points project through the surface of this organ, and 3) the presence of rows of spine-like hooks on the peduncle. The last-named structures like the haptoral spines are not cuticular in origin but arise in the parenchyma and in this regard resemble the anchors, haptoral hooks, and structures of the copulatory complex.

Type genus: *Rhamnocercus* n. g.

Rhamnocercus n. g.

Diagnosis.—Rhamnocercinae with spine-like hooks extending throughout length of peduncle. Two dorsal and two ventral clumps of haptoral spines situated bilaterally near the two pairs of anchors, accessory spines near midportion of haptor. Three haptoral bars, four eyespots, and twelve (or fourteen) haptoral hooks present. Parasites of marine fishes.

The closest generic relative of *Rhamnocercus* is problematical since it represents the type genus of a new subfamily. Differences and resemblances between Diplectaninae, the closest subfamily relative, and Rhamnocercinae, are given above.

Type species: *Rhamnocercus rhamnocercus* n. sp.

Rhamnocercus rhamnocercus n. sp.

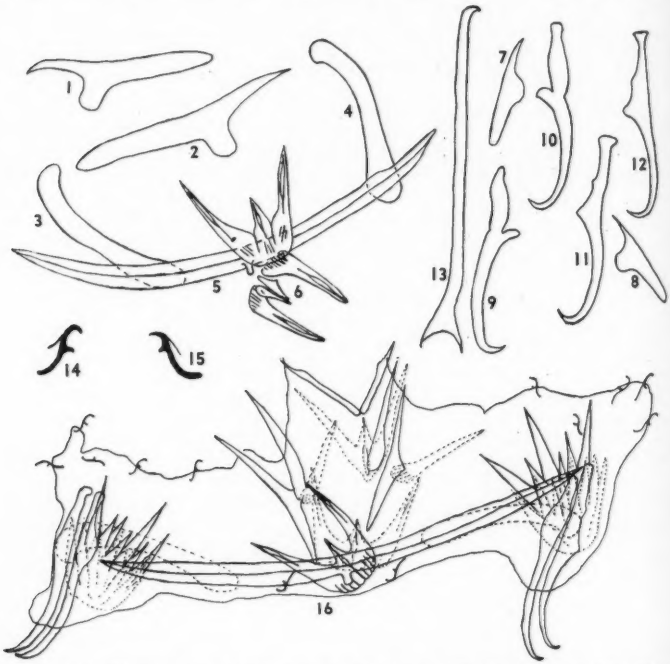
Host and locality—*Umbrina roncador* Jordan and Gilbert; Pacific Ocean, Scripps Oceanographic Institute pier, California.

Specimens studied—Twenty.

Type specimens—Type: U.S.N.M. Helm. Coll. No. 49425. Paratypes: U.S.N.M. Helm. Coll. No. 49426.

Description—Relatively small dactylogyrid with thin cuticle and relatively short, anteriorly directed cuticular body spines which occur as far forward as the level of the

vagina. Acicular internal spicules present in parenchyma of body proper and haptor. Total length 0.864 mm (0.630-1.260 mm), greatest body width 0.122 mm (0.090-0.144 mm) and usually present in anterior body half. Eyespots four, members of the posterior pair always larger and usually farther apart than those of the anterior pair. Accessory melanistic granules, apparently same type as those which comprise eyespots, often present in vicinity of eyespots. Granules comprising eyespots infrequently dispersed so as to give the impression that eyespots number less than four. Cephalic lobes 2 in number; head organs conspicuous (fig. 19). Pharynx subcircular to broadly ovate (transversely) in dorsal outline and 0.038 mm (0.032-0.044 mm) in greatest diameter. Peduncle relatively long, tapered posteriorly, and with rows of spine-like hooks along entire length (figs. 17, 18). Each peduncular hook composed of a relatively heavy shaft, an opposable piece and a point. Peduncular hooks diminished in length in anterior direction (figs. 1, 2, 7, 8). Haptor expanded laterally and almost twice as wide as greatest body width. Haptor length 0.037 mm (0.028-0.040 mm), greatest width 0.205 mm (0.180-0.234 mm). Three haptoral bars present; dorsal longest with pointed extremities, conspicuous longitudinal groove, and a characteristic projection on posterior border near midlength, length 0.166 mm (0.160-0.180 mm); two ventral bars of approximately equal size, articulated to dorsal bar and apparently represent remnants of a former, complete ventral bar; right ventral bar length 0.065 mm (0.060-0.072 mm), left ventral bar length 0.064 mm (0.060-0.068 mm) (figs. 3-5). Anchors similar in shape, approximately equal in size and possessing solid bases and regularly recurved shafts and points (figs. 9-12). Ventral anchor with superficial root



Figs. 1-16. *Rhamnocercus rhamnocercus* n. sp.—1, 2. Spine-like hooks at distal end of peduncle; 3, 4. Ventral bars; 5. Dorsal bar; 6. Accessory spines near midportion of haptor; 7, 8. Spine-like hooks at proximal end of peduncle; 9, 10. Ventral anchors; 11, 12. Dorsal anchors; 13. Cirrus; 14, 15. Haptoral hooks; 16. Dorsal view of haptor. Figs. 1, 2, 7, 8, 13-15, ×1600, others ×700.

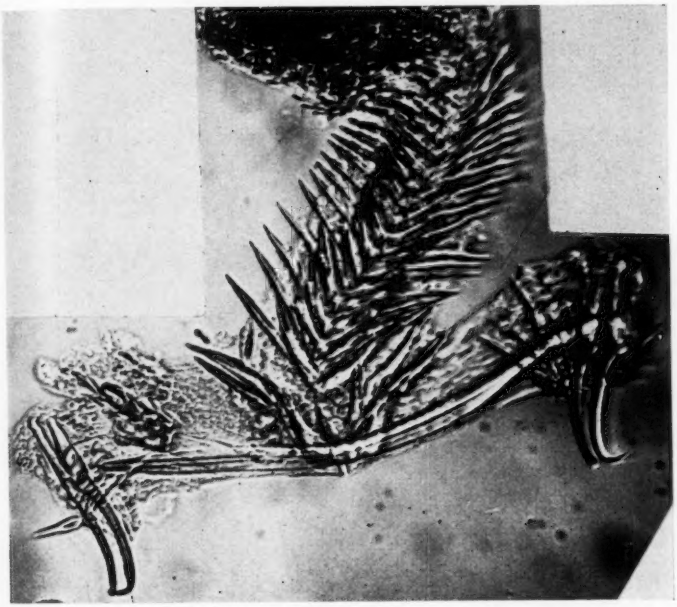
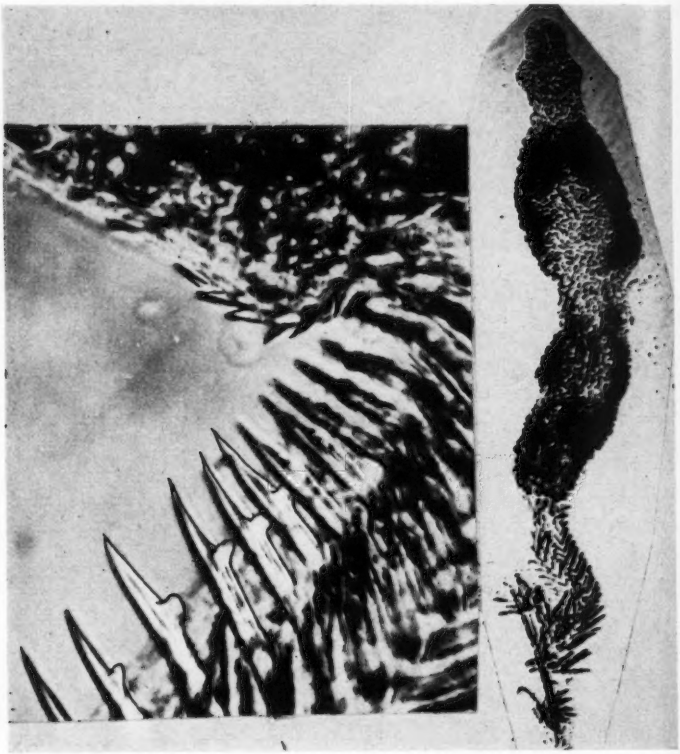


Fig. 17.—*Rhamnocercus rhamnocercus* n. sp. Haptor, peduncle, and distal end of body proper, ×675.

reduced but conspicuous, deep root well developed; ventral anchor length 0.072 mm (0.068-0.076 mm), greatest width of the base 0.031 mm (0.028-0.032 mm). Dorsal anchor with vestigial superficial root. Dorsal anchor length 0.071 mm (0.068-0.076 mm), greatest width of the base 0.029 mm (0.028-0.030 mm). Haptoral hooks 12 in number, approximately equal in size and normal in arrangement (Mizelle 1938); hooks of pair no. 1 unobserved; hooks of pair no. 5 situated on posteroventral haptoral border, hooks of other pairs shifted somewhat laterally and anteriorly (fig. 16). Each hook composed of a gently curved shaft, a sickle-shaped termination and opposable piece; hook base not differentiated as such. Posteriorly projecting process arising on sickle-shaped termination, usually conspicuous (figs. 14, 15). Hook lengths 0.010 mm (0.009-0.011 mm). Gonads with entire margins and situated in posterior body half, testis posterior. Vitellaria heavily developed and extend from rear margin of pharynx to anterior extremity of peduncle. Seminal vesicle well developed. Vagina on left lateral margin near body mid-length, surrounding cuticula wrinkled. Copulatory complex comprised of a cirrus, accessory piece lacking. Cirrus long, tubular, gently tapering distally, base expanded, tip recurved and directed posteriorly (fig. 13).

It is noteworthy that fresh specimens flown from California tended to lose the peduncular hooks and haptoral and body spines. Such material was frozen at the site of collection and upon arrival was apparently in good condition although the bottle in the vacuum container was broken en route. It is entirely possible that loss of such armament accompanies the early phase of degeneration of specimens.



Figs. 18, 19. *Rhamnocercus rhamnocercus* n. sp.—18. Enlarged photomicrograph of peduncle and distal end of body proper showing transition from spine-like hooks to scale-like spines, ×1400 (retouched); 19. Photomicrograph of whole mount, ×179.

REFERENCES

MIZELLE, J. D. 1938—Comparative studies on trematodes (Gyrodactyloidea) from the gills of North American freshwater fishes. Ill. Biol. Monogr. 17(1):1-81.

SPROSTON, N. G. 1946—A synopsis of the monogenetic trematodes. Trans. Zool. Soc. London 25:185-600.

New Strigeids from Minnesota Birds and Mammals*

Asa C. Chandler

Rice Institute, Houston, Texas

The parasites herein described were collected from birds and mammals examined in the parasitology laboratory at the University of Minnesota Biological Station. They include a new species of eared *Alaria* from skunks and cats, a tetracotyle from the subcutaneous fascia of a grouse, a new species of *Diplostomum* from the lesser yellowlegs (*Totanus flavipes*), and a species of *Ophiosoma* from the American bittern which is probably the same as a previously described species from this host, although there are some morphological discrepancies.

Alaria minnesotae n. sp.

During the summer of 1950 numerous specimens of an eared *Alaria* were found in the small intestine of each of four kittens which had been reared on a farm adjoining Itasca State Park, Minnesota. In the following summer some similar worms were found in the intestine of a skunk, *Mephitis mephitis,* in the same general locality, and a few immature and two sexually mature specimens were found in the intestines of some kittens that had been fed experimentally on some metacercariae of *Tetracotyle* type obtained from a ruffed grouse.

Since the development of the worms recovered from the kittens was about what would be expected in the time intervals after the experimental feedings it was at first suspected that they had developed from the grouse metacercariae that had been fed, but for reasons pointed out below, this is not a tenable conclusion. The grouse metacercaria is described below as a new species, *Tetracotyle bonasae,* until more is known about its life cycle.

Comparison of the *Alaria* from kittens and skunks with *Alaria canis* and other previously described members of the subgenus *Alaria* does not permit its assignment to any of these species unless the extent of variation from different hosts, and under varying conditions of fixation, is greater than it it now believed to be. It is therefore considered to be a distinct species, for which the name *Alaria* (*Alaria*) *minnesotae* n. sp. is proposed.

Description (measurements based on egg-bearing specimens only).—Body 1.45-1.9 mm. long, distinctly divided into fore- and hindbody; in living specimens hindbody often bent dorsad. Forebody 0.9 to 1. 25 mm. long (65 to 70% of total length) and 0.6 to 0.74 mm. wide, the hindbody 0.48 to 0.75 long and 0.46 to 0.68 wide, its width being, therefore, approximately equal to that of forebody and also to length of hindbody. Oral sucker weakly muscular, usually slightly broader than long, and uniformly slightly larger than ventral sucker; median diameter 105 to 114 μ. Pharynx very muscular, larger than either sucker, and distinctly pear-shaped, 118 to 155 μ long and 80 to 111 μ across broader posterior part. Ventral sucker usually broader than long, its mean diameter about 95 μ; usually situated less than its own diameter from posterior end of pharynx. Earlike appendages at either side of oral sucker capable of retraction; length when fully extended 150 to 185 μ. Holdfast organ 480 to 667 μ long and 248 to 310 μ wide, touching or partially overlapping ventral sucker with rare exceptions. Ovary transversely oval, situated at junction of fore- and hindbody. Vitellaria commonly extend forward to about level of posterior border of pharynx, dense in holdfast organ. Testes of irregular size and shape, usually bilobed; anterior one usually smaller, with either right or left lobe larger than

* Contribution from University of Minnesota Biological Station and Rice Institute.

other. Copulatory bursa about 110 to 125 μ in diameter, opening on dorsal side about 80 to 140 μ from posterior end. Eggs (in mounted specimens) 118 to 126 μ by 72 to 75 μ.

Hosts: *Mephitis mephitis* (type), and domestic cat.

Location: Small intestine.

Locality: Itasca State Park, Minn.

Type specimen: U.S.N.M. Helm. Coll. No. 37371; Paratypes U.S.N.M. Helm. Coll. No. 37372.

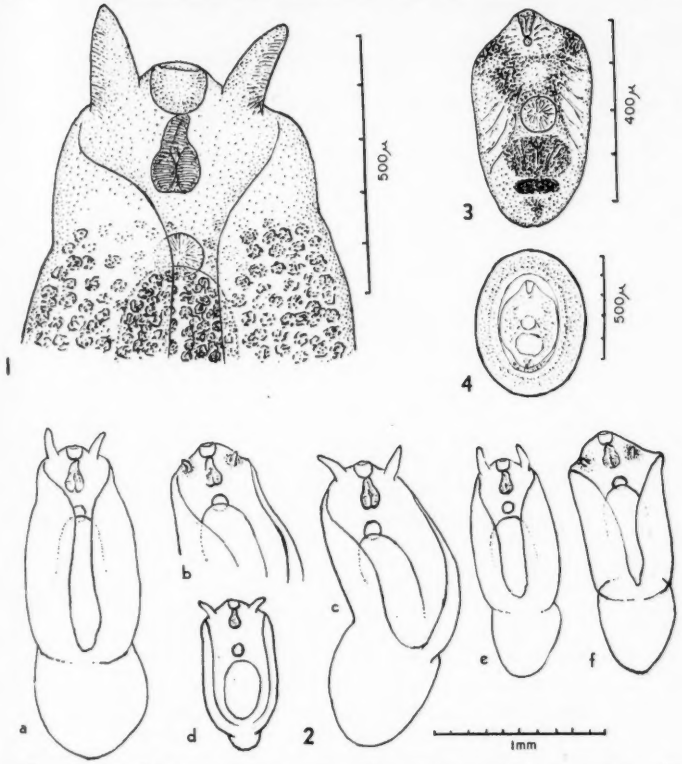
Alaria minnesotae is closely related to *A. alata* (Goeze, 1792), *A. canis* LaRue and Fallis, 1934, and *A. americana* Hall and Wigdor, 1918. *A. canis* was originally described from a dog in northern Ontario, but also occurs in the fox, *Vulpes fulva,* in the same locality and in *V. fulva alaskensis* in Alaska (Dubois and Rausch, 1950b). Some fox specimens from Ontario were reported by Swales (1933) as belonging to *A. alata,* but this was before *A. canis* was recognized as a distinct species, and they probably represent the latter species (see Dubois, 1938, p. 337). Mr. J. C. Pearson, of the Ontario Research Foundation, recently sent the writer numerous specimens of *A. canis* from a fox in northern Ontario, making possible a direct comparison of these as well as the type specimen of *A. canis* with *A. minnesotae.*

A. americana was first described from 4 of 300 dogs at Detroit, Michigan; it has subsequently been reported by Hall (1922) from a cat at St. Paul, Minn. (2 immature specimens); by Swales (1933) from the fox, *Vulpes fulva,* and the wolf, *Canis lycaon,* in Ontario; and by Douglas (1951) from a dog in California. *A. alata* is a European species which has not yet been reported with certainty from the Western Hemisphere although, as noted above, some specimens from Ontario that were probably *A. canis* were once recorded as *A. alata,* and Lutz (1933) tentatively referred to this species some specimens obtained experimentally in dogs and cats in Brazil, and also some found in the Brazilian fox, *Thoas cancrivorus.* As Lutz pointed out, this species might easily have been transported from continent to continent in domestic dogs or cats, and could readily establish itself since its intermediate hosts are cosmopolitan.

In addition to these species to which *A. minnesotae* is obviously closely related, *Alaria* (*Alaria*) *arisaemoides* also occurs in dogs and foxes in northern United States and Canada, and *Alaria* (*Paralaria*) *taxideae* has been reported by Erickson (1946) from both striped and spotted skunks, *Mephitis mephitis* and *Spilogale putorius,* in Minnesota, as well as from badgers, mink and weasels, and I have specimens which were collected by Rausch from a skunk (*Mephitis nigra*) in Michigan. This species, however, is easily differentiated by its lack of earlike appendages, broad forebody, and other characteristics.

A. minnesotae differs from *A. canis* in (1) its small size; (2) the shape of the forebody, which in *A. canis* is two to four times as long as broad, whereas in *A. minnesotae* it is somewhat less than twice as long as broad; (3) the holdfast organ, which is about the same width in both species but is 2½ to 4 times as long as wide in *canis,* and seldom as much as twice as long in *minnesotae;* (4) the pear-shaped pharynx; (5) the uniformly smaller size of the ventral sucker as compared with the oral sucker; and (6) the absence of thick muscular walls on the ejaculatory duct.

A. minnesotae differs from *A. alata* in its smaller size; the greater width of the holdfast organ relative to its length; the smaller ventral sucker relative



Figs. 1-4.—1. *Alaria minnesotae*, anterior end, ventral view; 2. *A. minnesotae*, outline sketches, ventral view, drawn to scale. a. From kitten experimentally fed cysts of *Tetracotyle bonasae;* uterus contains 8 eggs. b, c. From skunk, *Mephitis mephitis;* b. shows earlike appendages largely withdrawn; c. with appendages extended. d, e, f. From naturally infected kittens reared on farm near Itasca State Park; d. a very young specimen; e and f. young adults, e. with one egg in uterus, f. with none; f. has earlike appendages withdrawn; 3. *Tetracotyle bonasae* from subcutaneous fascia of ruffed grouse; 4. Cyst of *Tetracotyle bonasae* from subcutaneous fascia of ruffed grouse.

to the oral sucker; the greater length and pear shape of the pharynx; and the fact that the holdfast organ almost always touches and usually overlaps the ventral sucker, whereas in *A. alata* it almost never even touches it. From *A. americana*, *A. minnesotae* differs in shape of the forebody, which in *americana* is as broad as long and lyre-shaped; in having the ventral sucker smaller than the oral sucker, and situated in a more anterior position; and in having a more elongate holdfast organ.

Tetracotyle bonasae n. sp.

A ruffed grouse, *Bonasa umbellus*, accidentally killed by an automobile in Itasca State Park, was found to contain several hundreds of oval yellowish

cysts, about two thirds of a millimeter in length, in the loose connective tissue fascia covering the breast muscles, with a relatively small number in the fascia covering the thighs, and a very few over the lumbar muscles. A very small number was also found in the loose fascia between some of the individual breast muscles.

These cysts were found to contain strigeid larvae of the *Tetracotyle* type. The cysts are oval in shape and measure about 650 to 700 μ in length and 550 to 575 μ in diameter. The cyst wall appears to consist of concentric layers of different colors. The outer wall, presumably laid down by the host, is gray in color and about 80 to 100 μ in thickness, and is separated from the inner, yellowish, hyaline parasite cyst by a dark brown line; the parasite cyst is about 17 to 37 μ thick. The cyst cavity, which is almost filled by the enclosed metacercaria, is about 450 to 475μ long and 225 to 250 μ in diameter.

The metacercariae freed from the cysts measure about 450 to 575 μ in length and 275 to 350 μ in width. The body is rather flat dorso-ventrally, and broadest in its anterior third, with no demarcated hindbody. The oral sucker measures about 50 to 60 μ in diameter and may be considerably elongated in stretched specimens. The pharynx is very poorly developed, measuring only about 37 by 20 μ, with almost no musculature. The ventral sucker is relatively very well developed, measuring about 75 to 80 μ in diameter. In one stained specimen it measures 90 μ broad by 75 μ long. The holdfast organ is transversely oval, situated rather closely behind the ventral sucker, and measuring about 140 μ in breadth and 90 to 95 μ long. Immediately behind the holdfast organ is the genital rudiment, a transversely elongated, deep-staining organ measuring about 100 μ in breadth by 32 μ in length. There is no prepharynx or esophagus, and the intestinal ceca extend almost to the posterior end of the body, bending outward around the holdfast organ, and frequently having saclike enlargements at their posterior ends. The excretory pore is terminal. The reserve excretory system apparently consists of a coarse network of spaces, such as was described by Hughes (1927) for species of *Tetracotyle*. The irregularly defined spaces are filled with granules of varying size, some up to 10 μ in diameter, which move about freely, and are easily expelled from the excretory pore under cover-glass pressure. In stained specimens the portions of the body lateral to the ceca and also the areas lateral to the oral sucker, between this and the dense mass of tissue representing the lateral suckers, are divided into compartments by strands of parenchymal tissue.

From 50 to 100 or more of these cysts were fed, within a few hours after the death of the bird, to each of four half-grown kittens. These kittens had been brought to the University of Minnesota Biological Station from St. Paul and had been fed, over a period of about a month, on canned dog food, canned milk, and occasionally some cooked fish and bread. Since, however, the kittens were kept in an animal house to which various individuals, including children, could get access, it is not certain that the kittens could not have been fed on other food without our knowledge.

One kitten was autopsied on the fourth day after experimental feeding, and yielded five immature specimens of an eared *Alaria* which was easily recognized as belonging to the same species previously found in kittens and skunks, and described above as *A. minnesotae*. Another kitten, autopsied on

the 7th day, was negative. The remaining two kittens were autopsied on the 13th day; one was negative, and the other yielded two adult specimens of *A. minnesotae*, containing eggs.

The species of *Alaria* for which the life cycle has been completely worked out belong to the subgenus *Paralaria;* these have metacercariae of the *Diplostomulum* type, in which the reserve excretory system contains calcareous granules in vesicles at the ends of terminal branches. The only evidence concerning the life cycle of members of the subgenus *Alaria*, to which *A. minnesotae* belongs, is provided by Lutz (1933) who fed mesocercariae from a frog to two kittens and obtained unencysted metacercariae in the alveoli of the lungs, which when fed to another kitten and to a puppy developed into an eared *Alaria* very similar to *A. alata*, and referred to as "*Alaria* spec. (*alaria* ou *nattereri*)." Since the metacercariae in the grouse are of *Tetracotyle* type with freely moving granules, and also since there are striking differences in the size, relative and absolute, of the suckers and pharynx in these metacercariae as compared with the sizes of these organs in even the youngest *Alaria* obtained from the experimentally fed kittens, it must be assumed either that the kittens got access to some *Alaria* metacercariae from some extraneous source at about the same time they were fed the cysts from the grouse, or, less likely, that there were a few encysted *Alaria* metacercariae among the encysted *Tetracotyle* in the fascia of the grouse.

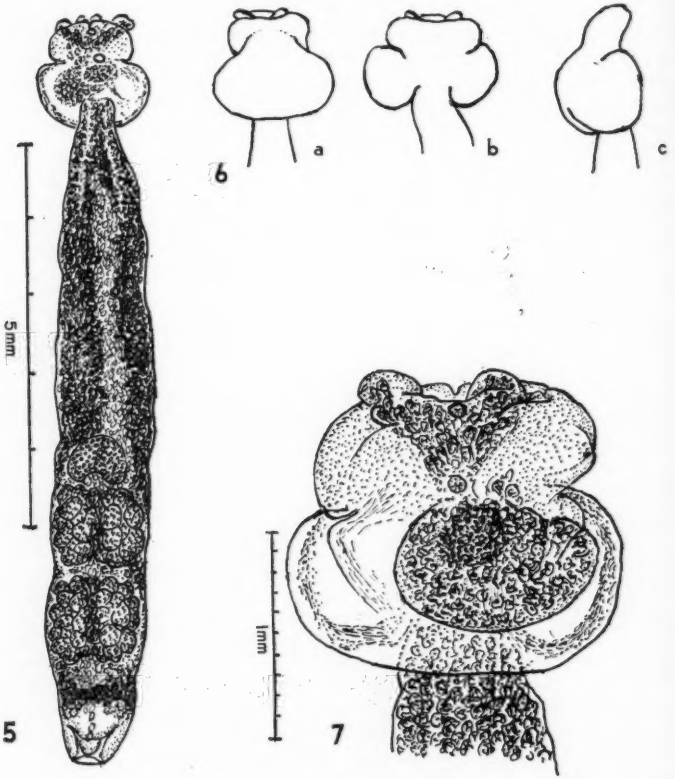
As far as the writer is aware, cysts containing larvae of *Tetracotyle* type have hitherto been reported from birds only by Lutz (1933) in Brazil. He fed mesocercariae with 8 gland cells, found in *Hyla crepitans*, to young chickens and pigeons and obtained *Tetracotyle* larvae similar to some which he had earlier found to develop into *Strigea vaginalis* in birds of prey, and also similar to *Tetracotyles* found in snakes, a fish, a fox, a mustelid, and a white mouse. All of these larvae developed experimentally into adults indistinguishable from *S. vaginalis*. Lutz (l.c.) also states that cysts of *Strigea falconis* var. *brasiliana* are encountered in abundance in aquatic birds, principally terns (*Sterna*). His figures of these cysts (Plate 3, fig. 4) show a striking resemblance to the cysts in *Bonasa umbellus* here described. It would not be surprising if *Tetracotyle bonasae* should prove to be the metacercaria of some species of *Strigea*, of which a number have been described from hawks and owls in northern United States (Chandler and Rausch, 1947; Dubois and Rausch, 1950a).

OPHIOSOMA sp., probably CRASSICOLE Dubois, 1948

Several specimens of a strigeid belonging to the genus *Ophiosoma* were found in the intestine of an American Bittern, *Botaurus lentiginosus*, collected at Waubun, Minnesota. An *Ophiosoma* from this host, *O. crassicolle*, was described by Dubois (1948) from four specimens collected by H. J. Van Cleave in Michigan. Our specimens resemble this species in many respects, but differ in a number of details. A description of the Minnesota specimens is, therefore, warranted; final decision as to whether Van Cleave's Michigan specimens and the Minnesota specimens represent the same species will have to wait until more material has been collected and studied from various parts of the country. It is possible that some of the discrepancies between Dubois'

description and the Minnesota specimens may be due to imperfection of the Michigan specimens. The Minnesota material consists of three whole mounts with total lengths of 9.5 to 10 mm. and one smaller sectioned specimen with a total length of 7.1 mm. (forebody 1.1 mm., hindbody 6.1 mm.). The measurements below are for the three larger whole mounts, since these are the only ones for which most of the measurements could be obtained.

Description.—Length 9.5 to 10 mm.; hindbody about 5.5 to 6 times length of forebody, to which it is attached by narrow neck. Forebody 1.43 to 1.48 mm. long and 1.48 to 1.65 mm. broad, divided into an anterior rectangular portion which is narrow anteroposteriorly and a larger posterior portion which is inflated laterally and ventrally. Hindbody 8 to 8.5 mm. long with width of 1.3 to 1.6 mm. in middle of posterior portion, whence it narrows to about 450 μ at truncated posterior end, and more gradually anteriorly to about same breadth at junction with forebody. Suckers poorly developed and not easily seen, both about 80 μ in diameter, the oral sucker about its own diameter behind anterior end, ventral sucker at level of constriction between anterior and posterior portions of fore-



Figs. 5-7. Ophiosoma sp., probably *crassicolle* Dubois, 1948. 5. Dorsal view; 6. Sketches of uncleared forebody. a. ventral, b. dorsal, c. lateral; 7. Forebody. Ventral view, with ventral wall of posterior part of forebody dissected away.

body. Pharynx small and indistinct. Holdfast organ with broad, rounded posterior lobe hanging free in inflated posterior part of forebody, and a smaller bifurcated anterior lobe, which may project slightly from cup of forebody at anterior end; all three lobes containing vitelline glands. Proteolytic gland situated on dorsal wall of cup of forebody, about 225 μ in diameter.

Vitelline follicles limited to holdfast organ in forebody, but occupying most of anterior half of hindbody to level of genital glands; limited to lateral regions and a narrow ventral band in region of ovary, and to narrow band ventral to testes, but forming a complete ring posterior to testes and anterior to copulatory bursa, broad ventrally and meeting narrowly dorsally. Ovary kidney-shaped, 850 to 980 μ broad and 550 μ long, situated slightly behind middle of hindbody, its anterior border almost at middle. Testes much lobed, occupying nearly full width of body and most of depth, except narrow band of vitellaria ventrally. Anterior testis situated immediately behind ovary, posterior testis separated by narrow space occupied by Mehlis' gland and transverse yolk duct. Both testes about same size, 1.05 to 1.35 mm. long and 1.2 to 1.5 mm. broad. Space between posterior testis and copulatory bursa occupied by swollen coils of vas deferens serving as seminal vesicle. Uterus extends forward almost to neck region, occupying central portion of body dorsal to vitellaria but ventral to genital glands, with numerous eggs. Copulatory bursa 575 to 590 μ broad proximally and 170 to 340 μ deep, with broad, rounded genital cone 170 to 250 μ long and 280 to 295 μ broad. Uterus and vas deferens meet in cone and open at tip. Eggs 115 to 120 μ by 68 to 70 μ.

One specimen deposited in U.S. Nat. Mus., Helm. Coll., No. 47837.

These Minnesota specimens differ from Dubois' description of *Ophiosoma crassicola* in (1) greater size, (2) shape of holdfast organ, (3) larger ovary, and (4) distribution of vitellaria in forebody where they do not form two small symmetrical masses as described by Dubois. These may be variations within the species, may be differences due to age, fixation, etc., or they may indicate a distinct species or subspecies. It should be noted that Dubois (1950) partially described an "Ophiosoma sp." from a single specimen from a Least Bittern, *Ixobrychus exilis*, from Ohio, which is about the same size as *O. crassicolle* but has a relatively shorter forebody, a more posteriorly located ovary, different shaped posterior testis, shallower copulatory bursa, and smaller eggs.

Diplostomum marshalli n. sp.

About two dozen specimens of a strigeid found in the intestine of a lesser yellowlegs, *Totanus flavipes*, proved to be a new species of the genus *Diplostomum*, for which the name *Diplostomum marshalli* is proposed, in honor of Dr. W. H. Marshall of the University of Minnesota. So far as the writer is aware only one other member of this genus has been found in Charadriiform birds, namely *D. vanelli* Yamaguti, 1935 from *Vanellus vanellus* in Japan.

Description.—Body divided into short, broad forebody, often broader than long but roughly circular in outline, and narrow, cylindrical hindbody commonly bent sharply dorsad at junction with forebody. Length 1.35 to 1.68 mm.; forebody thick-walled, 440 to 635 μ long and 550 to 775 μ broad, and hindbody 875 to 1150 μ long, and 400 to 440 μ in diameter. Oral sucker slightly broader than long, 60 to 90 μ long and 92 to 108 μ broad, flanked by two large, conspicuous, elongated pseudosuckers. Pharynx 50 to 60 μ in diameter, postero-dorsal to oral sucker. Ventral sucker 75 to 88 μ in diameter, concealed by holdfast organ. Holdfast organ large, broader than long, 155 to 250 μ long and 230 to 355 μ broad.

Vitelline follicles dense in neck region and posterior portion of forebody, in latter extending forward laterally to about level of middle of holdfast and into base of this organ, and extending posteriorly to posterior end of body, limited to median ventral band in region of reproductive glands, and expanding again to occupy most of width of body posterior to posterior testis, mostly in two lateral masses. Ovary broadly oval, about 125 to 160 μ broad and 100 to 125 μ long, situated slightly to right of midline of body anterior to middle of hindbody. Testes broader than long, halter-shaped with depression on

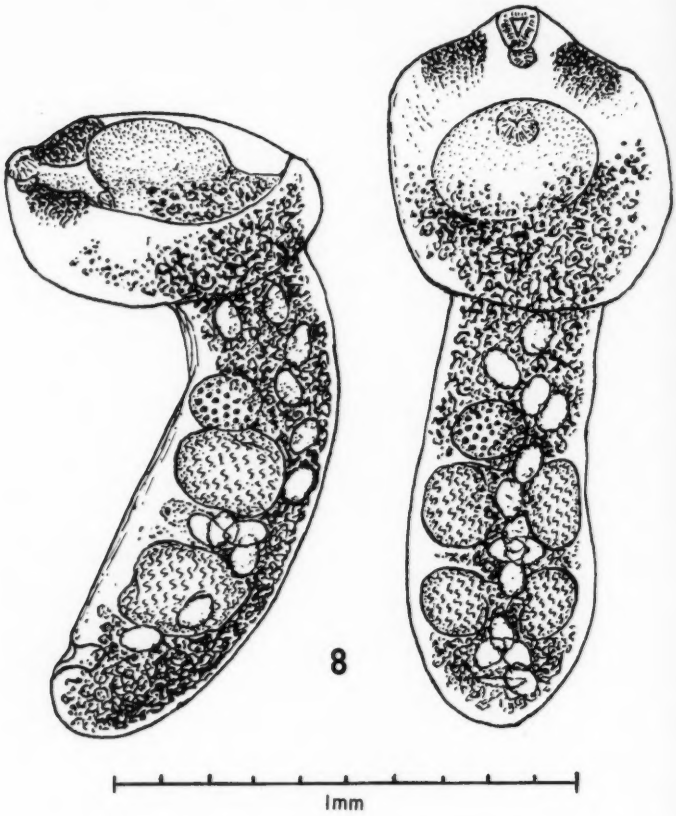


Fig. 8.—*Diplostomum marshalli;* left, lateral view; right, ventral view.

ventral side, variable in size, but occupying most of width of body. Anterior testis just behind ovary, posterior testis separated from anterior one, space between occupied by yolk reservoir, Mehlis' gland, and part of uterus. Uterus extends forward to near junction of forebody. Copulatory burst of moderate size, 100 μ or more from posterior end on dorsal side. Eggs large, 94 to 104 μ by 58 to 68 μ, numbering about 10 to 15 per fluke.

Host: *Totanus flavipes.*

Location: Intestine.

Locality: Waubun, Minnesota.

Type and paratypes: U.S. Nat. Mus. Helm. Coll. No. 47838.

This is the only species of Diplostomum thus far described in which the forebody is consistently as broad or broader than it is long.

REFERENCES

CHANDLER, A. C. AND R. RAUSCH 1947—A study of strigeids from owls in North Central United States. Trans. Amer. Micr. Soc. 66:283-292.

DOUGLAS, J. R. 1951—New parasite records from California dogs. Cornell Vet. 41:342-346.

DUBOIS, G. 1938—Monographie des Strigeida (Trematoda). Mém. Soc. Neuchât. Sci. Nat. 6:1-535.

——— AND R. RAUSCH 1948—Seconde contribution à l'étude des strigeides (Trematoda) Nor-Américains. Bull. Soc. Neuchât. Sci. Nat. 71:29-61.

——— AND ———1950—Troisième contribution à l'étude des strigeides (Trematoda) Nord-Américains. Ibid. 73:19-50.

——— AND ———1950a—A contribution to the study of North American Strigeids (Trematoda). Amer. Midl. Nat. 43:1-31.

ERICKSON, A. B. 1946—Incidence of worm parasites in Minnesota Mustelidae and host lists and keys to North American species. Amer. Midl. Nat. 36:494-509.

HALL, M. C. 1922—*Alaria americana* from the cat. J. Parasit. 9:43-44.

——— AND M. WIGDOR 1918—Two new flukes from the dog. J. Amer. Vet. Med. Ass'n. 53:616-626.

HUGHES, R. C. 1927—Studies on the trematode family Strigeidae (Holostomidae) No. VI. A new metacercaria, *Neascus ambloplitis* sp. nov., representing a new larval group. Trans. Amer. Micr. Soc. 46:248-267.

LARUE, G. R. AND A. M. FALLIS 1934—Morphological study of *Alaria canis* n. sp. (Trematoda: Alariidae), a trematode parasite of the dog. Trans. Amer. Micr. Soc. 55:340-351.

LUTZ, A. 1933—Considerações sobre o Distomum tetracystis Gastaldi e formas semelhantes, erroneamente chamadas agamodistomos. Mem. Inst. Oswaldo Cruz 27:33-49; German transl., 50-60.

SWALES, W. E. 1933—A review of Canadian helminthology. Canad. J. Res. 8:468-477, 478-482.

SZIDAT, L. 1928—Zur Revision der Trematodengattung Strigea Abildgaard. Zentralbl. Bakt., Orig. 105:204-215.

A Comparative Study of Detergent Effects on Ascarid Development*

B. J. Jaskoski

The Creighton University, Omaha, Nebraska

The resistance of ascarid eggs to varying conditions of temperature, humidity, and to various chemical agents is well known. Seamster (1950) and Jaskoski (1952) reviewed the pertinent literature and especially that concerning eggs of the swine ascarid. Ackert and Cauthen (1931) observed the effects of varied climatic conditions on the eggs of *Ascaridia galli*. Christenson et al. (1942) made a comparative study of the development of eggs of *Ascaridia galli* and *Heterakis gallinae*. They reported that eggs of *A. galli* were slightly less resistant to nicotine alkaloid than those of *H. gallinae*, but that *Heterakis* eggs had a lower thermal death point and appeared to be viable for longer periods of time. Hartman (1923) and Ackert and Cauthen (1931) observed that the eggs of *Ascaridia* from chickens were less resistant than the eggs of mammalian ascarids. Owen (1928), Taylor (1924), and Otto (1929) reported on the resistance of eggs of *Toxacara canis* and *Toxacara cati* under varied soil conditions and experimental laboratory conditions where temperature and humidity could be controlled. Eggs of the common cat and dog ascarids were reported to be generally more resistant to cold than to warm, dry temperatures. Lucker (1939) observed that certain halogenated hydrocarbons were lethal to the eggs of *T. canis*. Chlorosol and ethylene dichloride were found effective ovicides in the soil, the latter compound being more effective than CCl_4 or dichloropentane.

The nature of this resistance may best be sought by examination of the three egg membranes surrounding the ascarid ovum. Chitwood (1938) observed that the outer membrane is a protein, the middle membrane a chitinous supporting layer, and the inner a lipoid membrane. Ransom and Foster (1920), Zawadowsky (1928), and Hyman (1951) pointed out that the lipoid membrane is extremely important in regard to egg resistance and is impervious to all materials except lipoid soluble ones.

Since the chemical constituents of egg membranes of most ascarids are the same, it appeared timely to test the effects of chemical agents on the eggs of several of these species. The classification by Chitwood (1950), including *Heterakis* in the superfamily Ascaridoidea, is used here.

In preliminary work the author (1950) noted that certain commercial detergents exhibited a faster ovicidal effect if the two outer coats of the swine ascarid egg had been dissolved by a hypochlorite solution (anti-formin) before subjection to the detergents.

Acknowledgement.—The author gratefully acknowledges a generous supply of the detergents used from the Monsanto Chemical Company, the Union Carbide and Carbon Corporation, the Allied Chemical and Dye Corporation, and the E. I. DuPont de Nemours and Company. Appreciation is also expressed to Armour and Company, Omaha, for a supply of swine ascarids; to Dr. K. B. Kerr and Mr. W. C. McGuire of Dr. Salsbury's Laboratories for generous supply of chicken ascarids and heterakis used. Miss Jean Bonnema assisted in the experimental work.

* Aided by a Grant-in-aid from the Society of the Sigma Xi.

Materials and methods.—Species selected for the study were *Ascaris lumbricoides* var. *suum, Heterakis gallinae, Ascaridia galli, Toxacara canis,* and *Toxacara cati.* Eggs were procured from the swine ascarid by dissecting out terminal portions of the uteri according to methods outlined by the author (1952). The females of the four other ascarids were treated in a Waring blendor in a 0.2 per cent foramlin-tapwater solution until the eggs were freed from the uteri. The material was then strained through cheese cloth or 40 mesh screen which permitted the eggs to pass through, but retained larger bits of worm material. This method was found feasible and fast. Although eggs in different stages of maturity were present, it was easy to distinguish cleavage stages of fertilized eggs.

Eggs in baches of 500-2000 were incubated in stender dishes in 15 ml of a 5 per cent solution of each detergent and in control solutions of 0.2 per cent formalin-tapwater. Eggs remained immersed in the detergents at 31°C but were removed from incubation at 38°C at 12, 18, 24 and 28 or 36 hour intervals, filtered, washed, and re-incubated at 31°C. One additional experiment was done in which the protein and chitinous coats had been removed from the eggs with a hypochlorite solution. Because of great variations in development of ascarid eggs the detergent was considered effective only when it prevented development of *all* eggs in the batch.

The types, active chemical ingredients, manufacturers and designations of the detergents used, are as follows:

Detergent	Type	Active ingredient	Manufacturer
Areskap 100	anionic	monobutyl phenylphenol sodium monosulfonate	Monsanto
Aresket 300	anionic	monobutyl biphenyl sodium monosulfonate	Monsanto
Aresklene 375	anionic	dibutyl phenylphenol sodium disulfonate	Monsanto
Duponol 80	anionic	sodium n-octyl sulfate	Dupont
Nacconol NR	anionic	alkyl aryl sulfonate	Allied Chemical
Santomerse D	anionic	decyl benzene sodium sulfonate	Monsanto
Sterox CD	nonionic	polyoxyethylene ester	Monsanto
Sterox SE	nonionic	polyoxyethylene thioether	Monsanto
Tergitol NPG	anionic	alkyl aryl polyglycol ethers	Carbide and Carbon
Tergitol 08	anionic	sodium octyl sulfate	Carbide and Carbon

Development of controls.—Average and minimal developmental periods at 31°C for eggs of each species were observed as follows:

	Minimum	Average
A. suum	9 days	10 days
T. cati	5 days	6 days
T. canis	4 days	6 days
H. gallinae	4 days	5.5 days
A. galli	4 days	5.6 days

Because of the wide variations in development of ascarid eggs, no attempt was made to define a strict optimum temperature or time (in hours) for development. Since eggs were used from different worms from different host individuals in any one experiment, a fair cross-section of the period required for development should result. Temperatures of 31°-33°C appeared near the optimum for development of most eggs.

The author (1952) observed that de-coated eggs of *A. suum* were able to withstand a higher temperature in development than normal eggs. This was noted again here. The eggs of *T. cati, T. canis, A. galli* and *H. gallinae* developed to the motile-embryo stage at 38°C as well as about one-fifth of de-coated eggs of *A. suum.*

Detergent effects at 31°C.—A summary of the results of continuous exposure of ascarid eggs to detergent solutions at 31°C is presented in table 1. Duponol 80 was the only solution effective in preventing motile-embryo development in eggs of all of the five species tested. In each case, however, cleavage began but did not progress beyond the early morula stage. Nacconol NR was ovicidal before cleavage began in the case of eggs of *A. galli* in which there was a spreading and clearing of the protoplasm apparently because of entrance of the detergent. Sterox CD was ovicidal before cleavage began in the case of *T. canis* eggs, and prevented motile-embryo development in the eggs of *A. suum, T. cati,* and *A. galli.* Aresklene 375 produced the last-named effect on eggs of *T. canis* and *A. galli,* and Tergitol NPG on *A. suum* and *A. galli.* Of the remaining five detergents, two were effective only on one species each, *viz.,* Sterox SE on *A. galli,* and Santomerse D on *T. cati.* Areskap 100, Aresket 300, and Tergitol 08 did not prevent motile-embryo development in any of the five species.

Duponol 80 was thus the most effective agent used in these experiments. The eggs of *H. gallinae* were most resistant and those of *A. galli* were the least resisant to several detergents tested.

Detergent effects at 38 C.—Since 38°C is near the body temperature of most homoiotherms, the effect of detergents at this temperature was tested. Eggs were removed, washed, and replaced in formalin-tap water solution after exposure to the test solutions for 18, 24, 28 and also 36 hours in the case of eggs of *H. gallinae* which were the most resistant of those tested. A summary of the effects at 18, 24, and 28 hours is given in table 2. Increasing the temperature considerably enhanced the effectiveness of the detergents. In general, the some compounds that were effective at 31°C were effective at 38°C, with the

TABLE 1.—Detergent effects at 31°C. ME—motile-embryo, O—lethal effect

	DETERGENTS*									
	D	CD	NR	A3	NP	SE	SD	T	AR	A1
H. gallinae	O	ME	ME	ME	ME	ME	ME	ME	ME	ME
A. suum	O	O	ME	ME	O	ME	ME	ME	ME	ME
T. cati	O	O	ME	ME	ME	ME	O	ME	ME	ME
T. canis	O	O	ME	O	ME	ME	ME	ME	ME	ME
A. galli	O	O	O	O	O	O	ME	ME	ME	ME

DETERGENTS: A1—Areskap 100, AR—Aresket 300, A3—Aresklene 375, D—Duponol 80, NR—Nacconol NR, SD—Santomerse D, 34—Sterox CD, SE—Sterox SE, NP—Tergitol NPG, T—Tergitol 08.

addition of several which were ineffective at the lower temperature. The eggs of *H. gallinae* were most resistant and the eggs of *A. galli* the least resistant in all experiments. Lethal effect of the detergents was expressed in two ways. Either cleavage did not begin because of an early lethal effect, or cleavage was initiated and progressed to morula stages, but no motile embryos developed. These differences are indicated in table 2.

TABLE 2.—Detergents which prevented motile-embryo development at 38°C. * Indicates cleavage did not begin. See table 1 for abbreviations of detergents

Hours in detergent	*H. gallinae*	*A. suum*	*T. cati*	*T. canis*	*A. galli*
36	NR*				
28			NP*	A3* CD T	NP*, SD, SE, AR, A1
24			NR*		A3* AR* T
18	D	D*	D	D* NR*	NR* CD D

A summary of results of all experiments is presented graphically in figs. 1 and 2. An arbitrary "unit" of 4 was assigned to the eggs if they developed motile embryos in detergents at 31°C or after 18, 24, or 28 hours immersion at 38°C. Since ten detergents were employed, the best possible "score" would be 40. Figure 1 shows that the eggs of *H. gallinae* were most resistant with 36 units. Eggs of *A. suum* followed closely with 34, of *T. cati*, 29, of *T. canis*, 28, and *A. galli* with 15 units were least resistant to detergent action.

An arbitrary "unit" of 4 was likewise assigned to a detergent if it prevented motile-embryo development at the same temperatures and exposures noted. Since the eggs of the five species were tested under four conditions, the best possible "score" for a detergent would be 20. Duponol 80 was thus by far the most effective agent of the ten. Nacconol NR and Sterox CD had 9 units each. Areskap 100 was the least effective in all experiments.

An additional experiment was performed in which normal and de-coated eggs were used. Only with eggs of *A. suum* was there a significant difference between the resistance of normal and de-coated eggs. This was shown previously by the author (1952).

In order to ascertain whether or not effective detergents penetrated the egg membranes the detergents were made up in a 0.1 per cent solution of basic fuchsin. The dye solution alone did not penetrate the egg membranes, but penetration did occur when fuchsin was mixed with effective detergents since the egg protoplasm became permanently stained. Destruction of the eggs was evident from vacuolation (Duponol 80) and spreading of protoplasm (Nacconol NR).

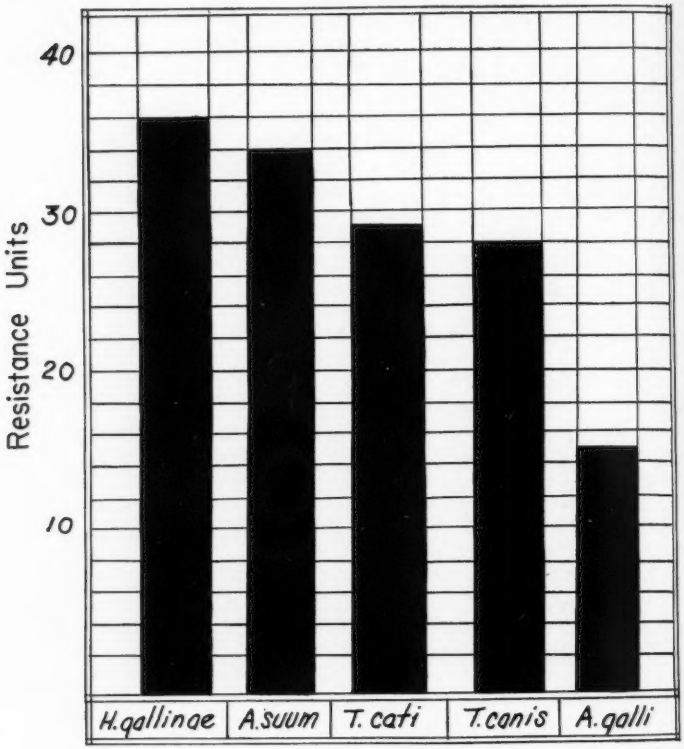


Fig. 1.—Comparative resistance of eggs to ten detergents at 31° and 38°C

Discussion.—Certain detergents of the nonionic and anionic types appear to be effective ovicides and larvicides. The mode of detergent action remains largely unknown. Though the active chemical ingredients must contribute to the activity, the complexity of detergent mixtures renders an analysis of mode of action very difficult. The present investigations show that effective detergents pass through the egg membranes as shown by the use of accompanying basic fuchsin. The most effective detergent used (Duponol 80) possesses a straight-chain compound of eight carbons with a sulfate radical. Nacconol NR, which was the second most effective of the ten tested, is an alkyl aryl sulfonate. Tergitol 08 which was comparatively ineffective is a sodium octyl sulfate, like Duponol 80, but the chain is branched.

There is also a difference in the visible effect on the egg protoplasm of different detergents. Duponol 80 causes a vacuolation and an internal "blistering" of the egg cell. Nacconol NR causes the protoplasm to spread and

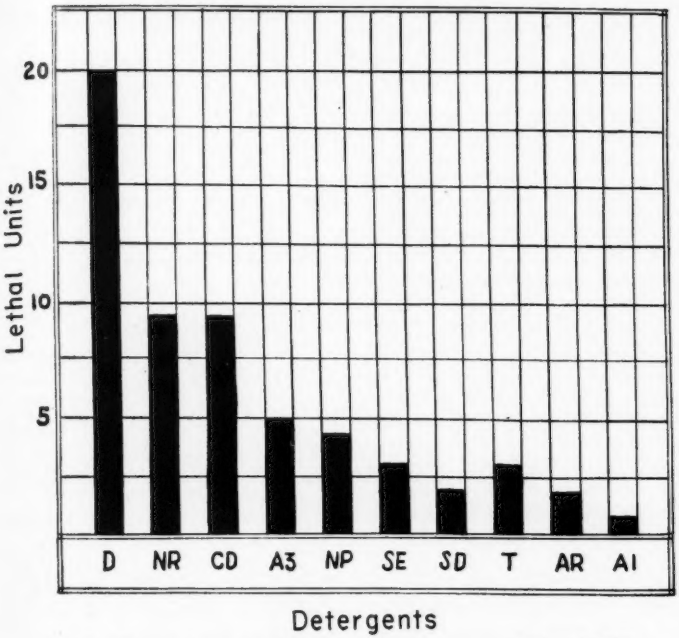


Fig. 2.—Comparative action of ten detergents against eggs at 31° and 38°C

assume a clear, glass-like appearance. Sterox CD is often effective in a manner that does not result in visible alteration of the egg structure.

As was expected, the eggs of closely related species were very similar in comparative resistance to detergent action (e.g. *T. canis* and *T. cati*). In general, the eggs of mammalian ascarids are more resistant than those of *Ascaridia galli*. The fact that the eggs of the chicken heterakis were decidedly more resistant than those of *A. galli* is perhaps suggestive of physiological differences between eggs of the two species.

The practical application of synthetic detergents as ovicides and larvicides remains to be determined. A suggested use is that of spreading agents for compounds such as phenol. The author (1952) observed that the addition of phenol greatly enhanced the ovicidal activity of Duponol 80. This was also true in preliminary trials with detergent Nacconol NR (unpublished results). In certain other trials (Aresklene 375) it was found that the addition of phenol actually prolonged the time necessary for ovicidal effect. Fleet (1945) observed that the addition of phenol to Nacconol NR did not improve the bactericidal action of the detergent. The phenol further lost the bactericidal action it exhibited alone. Fleet suggested that the detergent washed off the phenol. The suggestion is made here that the addition of phenol to a detergent like Aresklene 375 which has a phenolic ring as part of its structure may

result in formation of a complex quite different from the original, and one thus less effective.

SUMMARY

Eggs of five ascarid species (chicken, pig, dog, and cat) were subjected to solutions of ten detergents.

Immersion of ascarid eggs in detergents at 31°C was less destructive than 18-36 hour immersions at 38°C.

The eggs of *Heterakis gallinae* were the most resistant of those tested. Eggs of *Ascaridia galli* were least resistant.

Except in the case of *Ascaria suum* eggs, there was little difference in detergent action on normal or de-coated eggs.

The most effective detergents tested were Duponol 80 and Nacconol; the latter is an alkyl aryl sulfonate and the former, an n-octyl sodium sulfate.

Use of certain detergents as ovicides and larvicides or as spreading agents used in conjunction with ovicidal chemicals is suggested.

REFERENCES

ACKERT, J. E. AND G. E. CAUTHEN 1931—Viability of the eggs of the fowl nematode *Ascaridia lineata* (Schneider) exposed to natural climatic factors. J. Parasit. 18:113.

CHITWOOD, B. G. 1938—Further studies on nemic skeletoids and their significance in the chemical control of nemic pests. Proc. Helm. Soc. Wash. 5(2):68-75.

———1950—An introduction to nematology. Section I. 18-19.

CHRISTENSON, R., H. EARLE, R. BUTLER AND H. CREEL 1942—Studies on the eggs of *Ascaridia galli* and *Heterakis gallinae*. Trans. Am. Micro. Soc. 61:191-205.

FLEET, L. H. 1945—The antiseptic properties of surface active agents. Oil and Soap 22:245-249.

HARTMAN, E. 1923—Viability of the eggs of *Ascaridia*. Anat. Rec. 26:360.

HYMAN, L. H. 1951—The invertebrates: Acanthocephala, Aschelminthes, and Entoprocta. McGraw-Hill, New York. p. 410.

JASKOSKI, B. J. 1950—The role of the protein coat in the development of ova of *Ascaris lumbricoides* var. *suum*. J. Parasit. 36(6):Suppl.:33.

———1952—The protein coat in development of *Ascaris lumbricoides* eggs. Exper. Parasitol. 1:291-302.

LUCKER, J. T. 1939—The effect of some halogenated hydrocarbons on the eggs of *Toxacara canis*. Proc. Helm. Soc. Wash. 6:51-57.

OTTO, G. F. 1929—A study of the moisture requirement of the eggs of the horse, the dog, human, and pig ascarids. Amer. Jour. Hyg. 19(2):497-520.

OWEN, M. 1928—Survival of ova of *Toxacara canis* under natural soil conditions. J. Parasit. 15:143.

RANSOM, B. H. AND W. D. FOSTER 1920—Observations on the life history of *Ascaris lumbricoides*. Bull. U. S. Dept. Agric. No. 817.

SEAMSTER, A. P. 1950—Developmental studies concerning the eggs of *Ascaris lumbricoides* var. *suum*. Amer. Midl. Nat. 43(2):450-470.

TAYLOR, E. 1924—On the ascarids of the dog and cat. Ann. Trop. Med. Parasitol. 18:243-251.

ZAWADOWSKY, M. M. 1928—The nature of the egg shell of various species of *Ascaris* eggs. Trans. Lab. Exp. Biol. Zoopark, Moscow 3:116.

Statistical Methods and Techniques for the Measurement of Average Index Ratios of Ostracod Carapaces

Erhard M. Winkler
University of Notre Dame, Notre Dame, Indiana

Abstract: Height-Length Index ratios were measured with different methods, the projection method and the photographic method. The ostracod material, available for the measurements, consisted of the living ostracod *Cypricercus* sp. and the Pliocene *Cytheridea pannonica* Mehes from the Vienna Basin. Histograms show and explain the homogeneity of the available material and ground plans detect the inclusion of various molt stages. Possibilities of applications are mentioned.

Historical outline of the problem.—More than one hundred years ago A. Reuss published a monograph under the title *Die fossilen Entomostraceen des oesterreichischen Tertiaerbeckens* (The Fossil Entomostraceae of the Austrian Tertiary Basin). The book is still in use although many corrections have been made recently on the taxonomy, for which the shape, the markings of the carapace, and the pattern of the muscle scars are most important. Cooper, Sohn, Scott, Kesling and others discovered that the presence of early molt stages in the material caused confusion in describing new species and even new genera. Height/length measurements and determination of the height/length index ratio were introduced in the early forties of this century, and their use increased rapidly since. Measurements of the length and of the height of the valves disclose considerable variation in size and shape within one and the same species, but the height /length ratio or form ratio, expressed as an index number, eliminates the various sizes and limits attention to the roundness or the elongation of the valves. The normal distribution curve, or histogram, may still show a surprisingly great variety due to the inclusion of mutations and of material from different environments. Since measurements with the micrometer under the microscope are very time-consuming and hard on the eyes, easier techniques were devised after long experimentation.

Techniques of Carapace Measurement

Preparation of ostracod material for measurement.—Measurement of single valves is relatively easy, since they usually lie flat. A piece of strong cardboard is glued on a microscope glass slide with two to five circular holes punched in the central part of the cardboard. The specimens are placed in the holes with their contact margin resting on the glass slide. A cover glass is inserted into the cardboard, which is split for this purpose at the layer opposite the glass slide. Thus the specimens are enclosed between the glass slide at the base and the cover glass on top, permitting microscopic observations in both reflected and transmitted light. No more carapaces should be placed in one cell than can easily lie flat on the glass. Most octracodes are fossilized with both valves closed and cannot be placed with the fairly flat contact margin on the glass slide. If investigation of these in transmitted light is required, we must embed them in plastic or gum tragacanth with the contact margin accurately oriented parallel to the slide. This work, however, requires skill. Mounting the specimens on slides coated with paraffin is easier, but is possible only, if reflected light is sufficient.

Living ostracod material has to be treated with bleaching peroxide, in order

to remove the soft parts as completely as possible. Remains of the chitinous appendages, being very loosely attached to the muscle scars after the treatment, are easily cleaned out with a mounting needle. The opened valves are put into place on a glass slide with the contact margin facing the glass, the valves still being attached to each other by the dorsal ligament. In this case the glass slides are left entirely plain with no attached cardboard. The excess water is sucked off cautiously from around the valves and the set of valves is allowed to dry. No glue is necessary to fix the valves to their glass base, for the drying process apparently supplies enough calcite to guarantee natural bonding. Great care and manual experience are required in mounting the valves, since they are soft and easily deformable shortly after each molting stage. In a few cases the calcareous shell was found to be so weak that it could not support its own weight and collapsed. The measurements of the outlines are not influenced by such a collapse, however, for the contact margin adheres to the glass before the tumid shell collapses.

Measurements by the projection method.—The light beam of a strong microscope lamp (at least 75 watts) passes through the glass slide and valves as transmitted light; passes on through the microscope, is deflected 45° to a horizontal plane by one mirror and strikes a second mirror which causes it to fall on a piece of scale paper located on the working table parallel to the glass slide. The system of lenses should not magnify more than 20 diameters, since the projection itself enlarges the image considerably. A total enlargement of 50 times still assures clear outlines of the valves and a sufficient intensity of light for use in a darkened room. For the measurement of length and width of the valves, sharp outlines of the carapaces on the scale paper are required, while structural details of the shell may be neglected. The amount of total enlargement can be checked and adjusted quite easily with a millimeter scale by lowering or elevating the scale paper. With this method as many as 200 valves can be measured during a single hour of work.

Measurements by the photographic method.—Mounts of both fossil and living specimens are prepared as explained previously. The slide is ready for photography after a millimeter scale and a label are attached, the latter to prevent confusion in subsequent handling of prints. The use of a 35mm camera with a close-up attachment affording a relation of nearly 1:1 gives good results; the increase of the contrast with a microfile high-contrast film and various choices of background colors makes the outlines of the valves clear and facilitates measurement of the enlarged prints with calipers. Mistakes through duplication are avoided by crossing out the valves as they are measured on the prints.

Index ratios and their distribution curves.—The greatest height of the valve is divided by the greatest length of the carapace yielding the index ratio as a fraction which is expressed as percentage as the following example demonstrates: H-0.50mm, L- 1.00; H/L = 0.50, i.e. 50% H to L. Index numbers higher than 50% (hereafter written 50) and approaching 100 indicates increasingly round carapace; numbers close to 50 and below it show elongate form. Robert V. Kesling (1951) speaks of "Roundness and Elongation" of the valves.

The effectiveness of both the statistical and measurement methods was tried out with a living species of *Cypricercus* from a temporary glacial kettle pond near South Bend, Ind., and *Cytheridea pannonica* Mehes, of the Pliocene

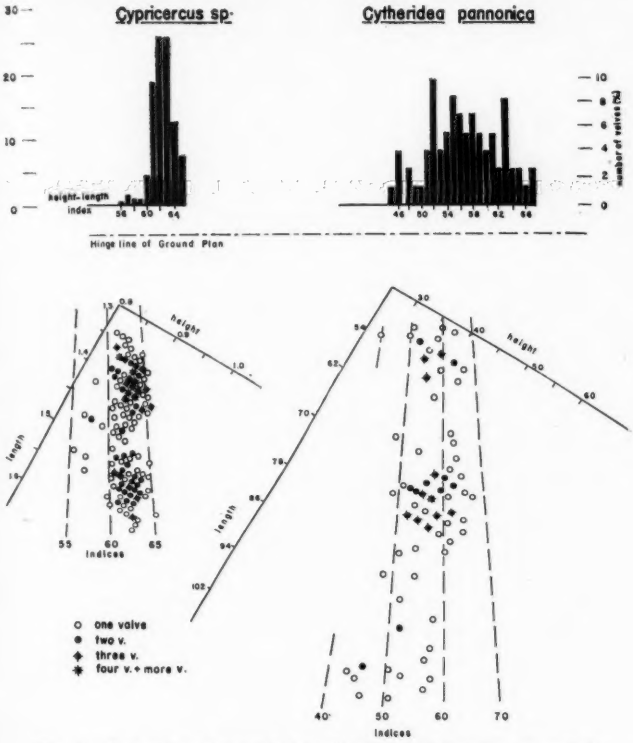


Fig. 1.—Side view and ground plan of the living *Cypricercus* sp. and the Pliocene *Cytheridea pannonica.*

freshwater strata of the Vienna Basin in Austria. The index ratios of valves from a single locality were plotted on a histogram with the index ratios (46, 47, 48, etc.) on the abscissus and the number of measured valves as percent on the ordinate axis. Figure 1 shows two histograms, of recent and fossil material. Since the material of the living valves was taken from a single locality at one time, the material should be entirely homogeneous. But the histogram still shows a fairly wide variety, although much less than that of the Pliocene *Cytheridea pannonica.* The lack of homogeneity revealed by this latter histogram is explained in part by the fact that the fossil valves were collected in a vertical range of more than 50 feet. Since drill cores, under normal circumstances, do not supply more than 100 valves of one species and depth, the data required for an ideal normal distribution histogram are not available. Curves with two and even three apices were not uncommon with *Cytheridea pannonica,* but those are not shown. However, a lack of homogeneity repeatedly occurring at the same spot on the curve may indicate a characteristic and not be due to a failure to fulfill the law of the big number.

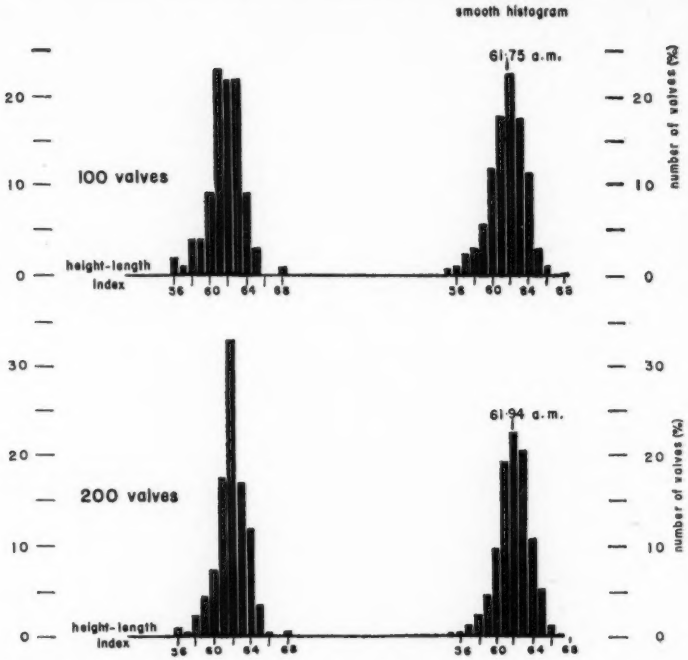


Fig. 2.—Histograms of left valves of the living *Cypricercus* sp. Presentation of crude and smooth histograms.

The arithmetical mean for comparing irregular curves or histograms with each other has proved most satisfactory. The arithmetical mean (a.m.) is secured by dividing the sum of all the index numbers by the numbers of valves measured. The influence of the law of the big number as well as the smoothing of a curve is well displayed in the figs. 2 and 3. The width of the histograms of the right and of the left valve of *Cypricercus* sp. is the same, and so is the shape of the histograms, but the left valves are more elongate. Histograms constructed on the basis of 200 valves show much smoother outlines than those of 100 valves and the smoother raw curves of 200 valves look very much alike. The a.m. of both the raw and the smooth curve are identical. The irregularity of the histograms can be explained clearly by the presentation of the ground plan of the histogram, i.e. the third dimension. A sharp break in the continuity of the ground plan proves the presence of two instars with *Cypricercus* and even three with *Cytheridea pannonica,* which increases its "elongation" with later instars. The changes of the index ratio with each molt stage will be discussed in another paper. The original intention was not to include various instars in one histogram, but the ground plan showed this to be an error. Sexual dimorphism may show two apices too; the ground plan, however, may clarify these results.

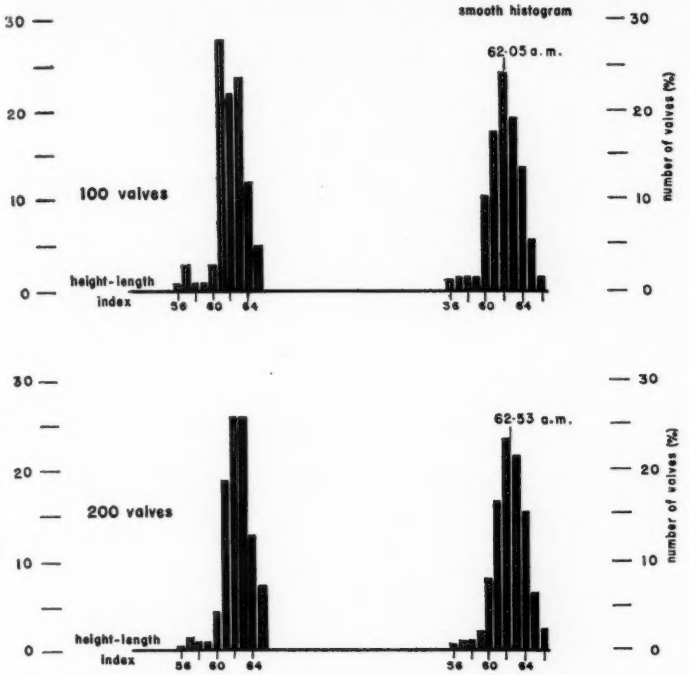


Fig. 3.—Same as fig. 2 but of right valves of *Cypricercus* sp.

These simplified techniques of measurement both by photographic and by projection, permit rapid and easy statistical investigations on populations of ostracods, fossil and living. Work with the a.m. gives a good basis for comparison of homogeneous populations. Dimorphism or the inclusion of young instars will be detected easily in the ground plan for each histogram. The range of the varieties within a histogram will be determined largely by the number of local varieties included in one sample. A separation of such local varieties is very difficult when dealing with fossil material. Investigations are under way to study such local varieties in the case of living ostracods in temporary ponds.

REFERENCES

HOFF, CLAYTON C. 1942—The ostracods of Illinois, their biology and taxonomy. Ill. Biol. Monogr. 19(1-2):1-196.

KESLING, ROBERT V. 1951—The morphology of ostracod molt stages. Ibid. 21(1-3):1-324.

SCOTT, HAROLD W. 1944—Muscle scar pattern on some upper paleozoic ostracods. Jour. Paleon. 18:162-171.

———1951—Instars and shell morphology of Eoleperditia fabulites. Ibid. 25:321-326.

SOHN, ISRAEL G. 1950—Growth stages in fossil ostracods. Am. Jour. Sci. 248:427-434.

———1950—Growth series of ostracods from the permian of Texas. U.S. Geol. Survey Professional Paper 221-C. pp. 33-43.

The Specific Validity of Epischura massachusettsensis Pearse (Copepoda: Calanoida)

Arthur G. Humes

Boston University, Boston, Massachusetts

The original description of *Epischura massachusettsensis* Pearse, 1906 was based upon nine females collected at Wellesley, Massachusetts, on April 20, 1905. The fact that this species has since then been known only from the brief description of the few original females has led some authors (for example, Marsh, 1933) to regard the species as of uncertain validity. A study of recently collected specimens has shown that it is a distinct and valid species.

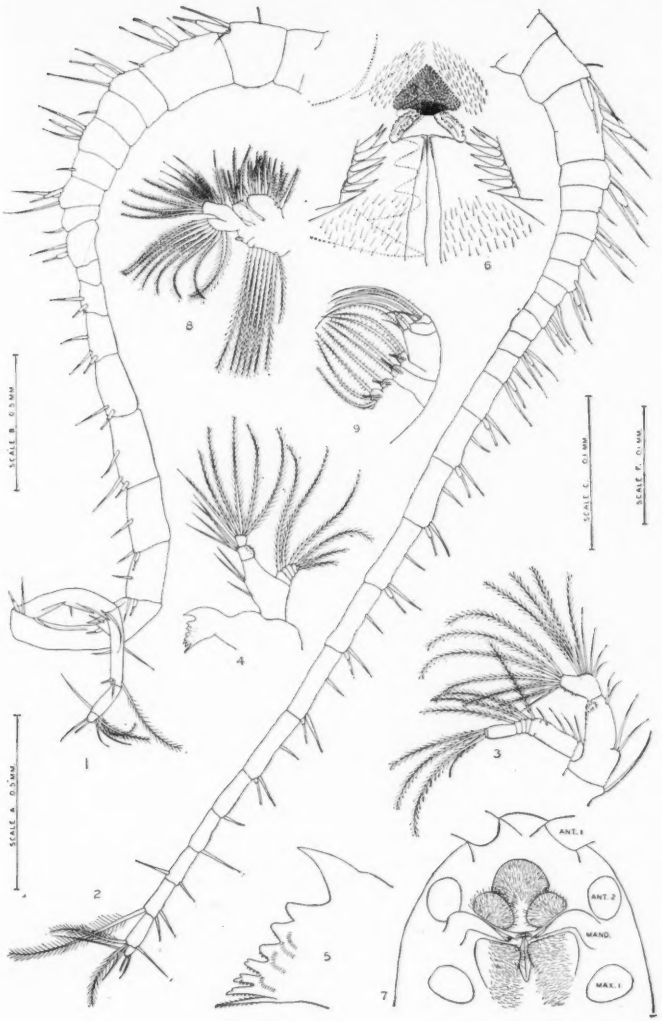
Both sexes of this copepod were collected in large numbers on May 10 and 17, 1952, in a shallow pool about forty feet in diameter at Dover, Massachusetts. The females were without egg sacs, although many had single spermatophores attached. The water temperature on these two days was 14°C. Specimens of both sexes have been deposited in the U. S. National Museum.

Male.—The average length (with extremes) of ten adult males is 3.13 mm (2.92-3.34 mm), measuring from the anterior end of the head to the posterior end of the caudal rami. Specimens preserved in 70% alcohol are reddish, except for a colorless or slightly yellowish band around the body between the insertions of the second antennae and the maxillipeds. The right first antenna (fig. 1) is constricted at the tenth segment and geniculate between the eighteenth and nineteenth. The left first antenna (fig. 2) is unmodified and reaches approximately to the base of the caudal rami. The second antennae (fig. 3) have the general form of other species in the genus.

The mandibles (fig. 4) possess a masticatory lobe with well-formed teeth (fig. 5) and a large biramous palp. The mouth (fig. 6) is bounded anteriorly by a triangular verrucose area and laterally by elliptical roughened areas and longitudinal rows of six teeth, the most anterior tooth in each row being bifurcated apically. Anteriorly and posteriorly to the mouth region there are projecting lobes forming the lips (fig. 7). The anterior lip consists of three swollen lobes, one median and two lateral, covered with fine, hair-like setae, easily seen in a lateral view of the entire copepod. The posterior lip is formed of two similarly setiferous but less prominent lobes situated behind the mouth area. The masticatory lobes of the mandibles lie between the two lips, with the rows of teeth placed vertically.

The first maxillae (fig. 8), second maxillae (fig. 9), and maxillipeds (fig. 10) are in general respects similar to those described for other species of the genus. The four pairs of swimming legs resemble those of other species except for certain details of armature. The first swimming leg (fig. 11) shows groups of fine setae near the outer edge of the first basipodite. The second (fig. 12) and third (fig. 13) swimming legs possess short spines instead of fine setae on the outer edge of the first basipodite and have a row of fine setae along the inner edge near the large internal seta. On the second basipodite the row of fine setae present on the outer edge in the first leg is absent and appears instead along the inner edge of this podomere. The fourth swimming leg (fig. 14) is like the second and third except for the absence of the single large seta and the row of fine setae on the inner edge of the first basipodite.

The structure of the fifth pair of legs is very characteristic. The left fifth leg (fig. 16) bears on the second basipodite an outer distal seta and an inner digitiform lobe whose length is about equal to the width of the second basipodite. The proximal podomere of the exopodite bears an outer distal seta. The distal podomere is armed with four spine-like setae, three along the outer margin and one terminal. In addition, the distal podomere possesses three rows of fine, hair-like setae as indicated in the figure. The right fifth leg (fig. 17) has an elongated basipodite bearing an outer distal seta (often difficult to detect and not shown in the figure) and on the inner distal edge a short digitiform process only about one-half as long as the corresponding lobe on the left leg. The exopodite consists of



Figs. 1-9. *Epischura massachusettsensis*.—Male: 1-3. Antennae. 1. Right first; 2. Left first; 3. Second; 4. Mandible; 5. Distal end of masticatory lobe of mandible; 6. Mouth region as seen in flattened glycerine mount, with dashed line indicating position of masticatory lobe of mandible on right side; 7. Ventral view of head region showing anterior and posterior lips. 8, 9. Maxillae. 8. First; 9. Second. Drawn with the aid of a camera lucida. Scale A applies to figs. 1 and 2, scale B to 3, 4, 7-14 and 19, scale C to 6, and scale F to 5.

an apparently single, strongly flexed podomere. There is an inner seta near the point of flexure. Just distal to the seta there is a slight indication of an articulation. Beyond this point the podomere becomes somewhat flattened on the side facing the digitiform lobe. The edge here has the appearance of being segmented. In this region there are four minute spines arranged as shown in the figure.

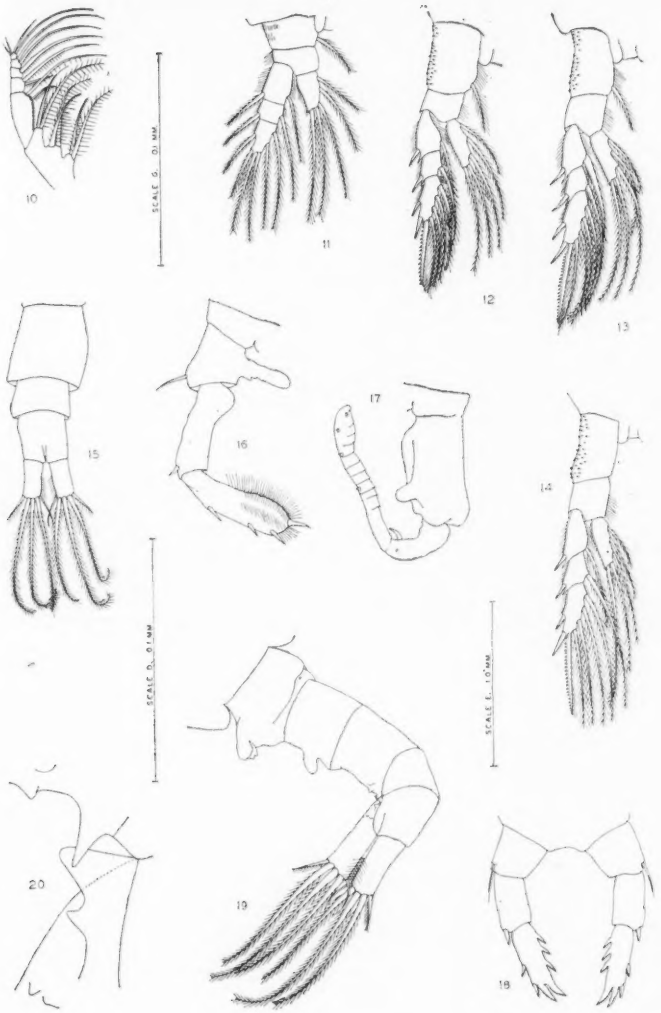
The 4-segmented abdomen (fig. 19) is strongly flexed to the right. The genital somite (sixth thoracic) bears on the right side a conspicuous process having an irregular ridge distally. On the right side of the first abdominal somite there is a short blunt lobe. The right border of the third abdominal somite is much shorter than the left, so that the abdomen is sharply bent at that level. The processes on the right side along the second, third, and fourth somites are indicated in detail in fig. 20. The fourth abdominal somite is shortened on the right side so that the outer border of the right caudal ramus is about one-third longer than the inner edge.

Female.—The average length of ten adult females is 3.40 mm (3.27-3.55 mm). The color of alcoholic specimens is like that of the males. The first antennae are similar to the left first antenna of the male. The remaining appendages are essentially like those of the male except for the fifth pair of legs (fig. 18). In these the second basipodite bears a seta near the outer distal corner. The exopodite consists of two podomeres, the first bearing an outer distal spine-like seta. The second podomere bears seven setae or spines, arranged as follows: one spine-like seta approximately at the beginning of the distal third of the outer edge, a similar seta at the outer distal corner, an indistinctly articulated seta internal to and larger than the one just mentioned, a somewhat angular spine at the inner distal corner, and three equidistant smaller spines on the inner edge. All seven spines (or setae) have a row of very fine spines along one edge, as shown in the figure.

The straight 2-segmented abdomen (fig. 15) lacks the processes present on the male abdomen. The caudal rami are like the left caudal ramus of the male.

Remarks.—The fifth legs in both sexes in *Epischura* are so characteristic that it is readily possible to distinguish the several species on the basis of these appendages alone. The seven known species, omitting the doubtful *E. fluviatilis* of Herrick (1883), are *E. lacustris* Forbes 1882 (including var. *columbiae* Forbes 1893), *E. nevadensis* Lilljeborg 1889, *E. nordenskiöldi* Lilljeborg 1889, *E. baikalensis* Sars 1900, *E. massachusettsensis* Pearse 1906, *E. chankensis* Rylov 1928, and *E. udylensis* Borutskii 1947. Smirnov (1936) has proposed the subgenus *Epischurella* for the Palearctic *E. baikalensis* and *E. chankensis*. Probably *E. udylensis* belongs here also. The remaining four North American species remain in *Epischura s. str.* In males of all but *E. massachusettsensis* a curved, rather pointed process about as long as the two exopodite podomeres together is located on the inner side of the second basipodite of the left fifth leg. The corresponding process in *E. massachusettsensis* (fig. 16) is relatively short and digitiform.

In the female the armature of the second podomere of the exopodite of the fifth pair of legs varies interspecifically. In *E. lacustris*, according to the original description of Forbes (1882), there are six spines, four of them terminal and one on each side. Schacht (1898) mentions seven spines, however, in the type specimens, three on the outer margin, three apically, and one on the inner edge. In specimens of this species from Lake Erie (USNM 62574) I find seven spines thus arranged. The podomere is relatively long and slender, being about four times longer than wide, not including the spines. In *E. nevadensis* the normal number of spines seems to be six, with four of them terminal, but there are known to be occasionally seven spines (Forbes, 1893) or in a single instance eight (Marsh, 1933). In *E. nordenskiöldi* there are five spines (occasionally six according to Schacht, 1898) all near the distal end. In *E. baikalensis* there are three spines along the outer edge. In *E. chankensis* and *E. udylensis* the exopodite is short and broad with three sub-



Figs. 10-20. *Epischura massachusettsensis*.—10. Male, maxilliped. 11-14. Male, swimming legs. 11. First; 12. Second; 13. Third; 14. Fourth; 15. Female, genital somite and abdomen; 16. Male, left fifth leg; 17. Male, right fifth leg; 18. Female, fifth pair of legs; 19. Male, genital somite and abdomen; 20. Male, detail of processes at flexure of abdomen. Drawn with the aid of a camera lucida. Scale D applies to figs. 16-18, scale E to 15, and scale G to 20.

terminal spines. In *E. massachusettsensis* there are seven spines arranged much like those in *E. lacustris*, with three terminal, three on the outer edge, and one on the inner margin (fig. 18). The podomere is much stouter, however, being only about twice as long as wide, and the spines are more robust.

REFERENCES

BORUTSKII, E. V. 1947—Concerning a new species of the genus *Epischura* from the Amur basin (*Epischura udylensis* sp. n., Copepoda-Calanoida). In Russian. Dokl. Akad. Nauk SSSR 58:1513-1515.

FORBES, S. A. 1882—On some Entomostraca of Lake Michigan and adjacent waters. Amer. Nat. 16:537-542 and 640-649.

———1893—A preliminary report on the aquatic invertebrate fauna of the Yellowstone National Park, Wyoming, and of the Flathead region of Montana. Bull. U. S. Fish Com. for 1891:207-258.

HERRICK, C. L. 1883—Heterogenetic development in *Diaptomus*. Amer. Nat. 17:381-389 and 499-505.

LILLJEBORG, W. IN J. DE GUERNE AND J. RICHARD 1889—Revision des Calanides d'eau douce. Mém. Soc. Zool. France 2:53-181.

MARSH, C. D. 1933—Synopsis of the calanoid crustaceans, exclusive of the Diaptomidae, found in fresh and brackish waters, chiefly of North America. Proc. U. S. Nat. Mus. 82:1-58.

PEARSE, A. S. 1906—Fresh-water Copepoda of Massachusetts. Amer. Nat. 40:241-251.

RYLOV, W. M. 1928—Über eine neue *Epischura*-Art aus dem fernen Osten (*Epischura chankensis* sp. nov.; Copepoda, Calanoida). Zool. Anz. 77:125-137.

SARS, G. O. 1900—On *Epischura baikalensis*, a new calanoid from Baikal Lake. Ann. Mus. Zool. Acad. Imp. Sci. St. Petersbourg 5:226-238.

SCHACHT, F. W. 1898—The North American Centropagidae belonging to the Genera *Osphranticum*, *Limnocalanus*, and *Epischura*. Bull. Illinois State Lab. Nat. Hist. 5:225-269.

SMIRNOV, S. S. 1936—*Epischura baikalensis* G. O. Sars (Crustacea Copepoda) from Kamtshatka. In Russian with German summary. Trav. Inst. Zool. Acad. Sci. URSS 3:59-65.

Entocythere occidentalis sp. nov., a Cytherid Ostracod Commensal on Western Species of Pacifastacus

Eugene N. Kozloff and Donald C. Whitman
Lewis and Clark College, Portland, Oregon

In the course of examination of specimens of crayfishes referable to *Pacifastacus leniusculus* (Dana), *P. trowbridgii* (Stimpson), and *P. klamathensis* (Stimpson)* for commensal organisms, a new species of *Entocythere* was found in association with *Entocythere columbia* Dobbin (1941). *E. columbia* is the only species which has been described from crayfishes collected in the Pacific Coast states. The localities given by Dobbin are in Washington; one is recorded only as "Columbia River," but the other three are more definite and are in the western part of the state. The host species was not designated by Dobbin.

Entocythere columbia and our new species, for which we propose the name *Entocythere occidentalis*, were found to occur on nearly all larger individuals of the three species of *Pacifastacus* which we examined from several localities in Multnomah and Clackamas counties, Oregon. Both of these ostracods occur principally among the cuticular hairs on the ventral side of the thorax of the host, but a few are found among the mouth parts and on the branchiae.

Type specimens of *Entocythere occidentalis* were taken from crayfishes referable to *Pacifastacus trowbridgii* collected in Crystal Springs Creek, Portland, Multnomah County, Oregon, March 6, 1952, by the senior author. The holotype (male), allotype (gravid female), and paratypes (two copulating pairs, one gravid female) have been deposited in the United States National Museum (U.S.N.M. Nos. 93503 to 93507).

Methods.—Although *E. columbia* and *E. occidentalis* are usually abundant on the host and may easily be removed alive by means of fine-tipped forceps, we found that collection of living ostracods is facilitated by placing the host in a dish of water in which a few small crystals of chloretone have been dissolved. As the ostracods are narcotized they fall off the host and may be transferred by a pipette from the bottom of the dish to the fixing reagent. For fixation we used 95% ethyl alcohol. Hoff (1942) described a method of dislodging and fixing the ostracods in one operation by placing the host crayfish directly into 95% alcohol. This procedure is rather effective, but in our experience a considerable proportion of the ostracod population tended to remain attached to the host.

Subsequent to fixation most of the specimens were dehydrated in absolute alcohol, cleared in toluol, and mounted in Harleco synthetic resin. Some of

* Bott (1950) established the genus *Pacifastacus* for the crayfishes of the subfamily Astacinae occurring in western North America, and designated *Astacus klamathensis* Stimpson as the genotype. The taxonomy of western crayfishes is not well understood and is in need of revision. The three species occurring in the vicinity of Portland, Oregon show considerable intergradation, but for the present it seems best to apply separate specific names to examples which conform closely to the original descriptions.

the specimens were stained lightly in acid fuchsin before dehydration. A part of the material was cleared by gradual replacement of alcohol by glycerin, and mounted in glycerin jelly.

Entocythere occidentalis sp. nov.

Figs. 1-13

Male.—The valves of the shell (fig. 1) are oval, with the anterior, posterior, and dorsal margins rounded. The ventral margin shows no appreciable curvature. The height of the shell is greatest at about the beginning of the posterior third of its length. In twenty copulating males taken at the type locality on five *Pacifastacus trowbridgii*, the length of the shell ranged from about .38 mm to about .40 mm, and the height ranged from about .21 mm to about .22 mm. There are a number of very fine setae distributed over the outside surface of each valve. These are more noticeable along the ventral and end margins. There is no pigmentation in the valves of the shell, but granules of brown pigment are irregularly dispersed in the animal itself, especially in the more superficial layers of the dorsal and lateral areas. The median eye is heavily pigmented and is situated dorsally near the end of the first one-fifth of the body. In living individuals the outline of the eye, as seen from the lateral aspect, is oval. The shape is often distorted in specimens in permanent preparations.

The antennules (fig. 4) are composed of six podomeres. The first podomere is the longest, and the third is the shortest. The second, fourth, fifth, and sixth podomeres are approximately the same length. There is one seta on the first podomere, one on the second, two setae on the third, six on the fourth, none on the fifth, and five terminal setae on the sixth podomere.

The antennae (fig. 5) are composed of four podomeres, the third of which is divided. The basal podomere bears a long flagellum-like seta which extends a short distance beyond the tip of the dorsal claw. There is one seta on the second podomere. The proximal division of the third podomere bears two setae which are approximately equal in length. There is a single seta originating near the middle of the distal division of the third podomere. Of the three terminal claws, the ventral one is longest. It is slender, tapers gradually from its base, and is curved ventrally at its tip. It bears about twelve comb-like teeth in its distal half. The dorsal claw (fig. 6) is stout and bears about fourteen teeth on its flattened terminal portion. The middle claw is about one-half the length of the ventral and dorsal claws and bears about thirteen teeth.

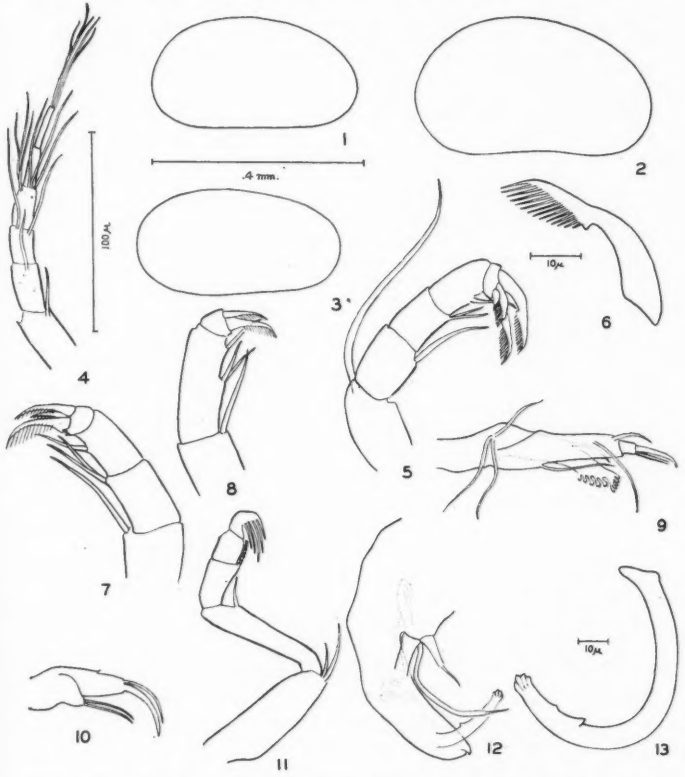
The distal end of the protopodite of the mandible (fig. 9) has six teeth. The most distal tooth is wider than the others and has five cusps. The more proximal teeth become progressively smaller; each appears to have three cusps, although the cusps are poorly developed on the last two teeth. There is a single seta on the convex surface of the protopodite. The respiratory plate of the mandible is represented by three long setae. The only evidence of segmentation in the mandibular palp is the demarcation of the distal podomere. A long seta extends from the end of the basal one-third of the palp on its flexor side. Another long seta originates on the lateral surface of the palp at about the end of the third quarter of its length. A shorter seta originates on the extensor side of the palp a short distance proximal to the demarcation of the distal podomere. The distal podomere bears a heavy spine-like seta and two slender setae.

The respiratory plate on the proximal portion of the maxilla bears about eighteen setae. The protopodite (fig. 10) bears two long setae. The palp is unsegmented and terminates in two setae of approximately equal length. The more ventral of these setae is relatively heavy and spine-like.

The three pairs of thoracic legs are similar, although the legs of each successive pair are slightly longer than those of the preceding pair. Each leg (fig. 11) is composed of four podomeres. The anterodistal corner of the first podomere of the first leg bears two setae, one of which is about twice as long as the other. There is only one seta in the corresponding position on the second and third legs. The second podomere bears a single seta. The last two podomeres are indistinctly separated from one another. The third bears a short, minutely hirsute seta which is about equal in length to the fourth podomere. The terminal claw bears five teeth.

The basal piece of the copulatory complex (fig. 12) consists of a broad proximal portion from which the clasping apparatus and the ventral and dorsal fingers originate,

and a more narrow distal portion. There is a prominent incision at the distal end of the basal piece. The sperm duct may be traced to a structure in the distal half of the basal piece which apparently serves as the penis. The spine at the tip of the ventral finger extends beyond the tip of the basal piece. The dorsal finger is only about one-half as long as the ventral finger, but its spine is about the same length as the spine of the latter. The clasping apparatus (fig. 13) is C-shaped, its outer edge being rather smoothly curved. The distal end is divided into four rather well-marked teeth. A prominent tooth is located on the inner border of the clasping apparatus near the beginning of its distal one-third, and there is a smaller tooth about half way between the first tooth and the distal end.



Figs. 1-13. *Entocythere occidentalis* sp. nov.—1-3. Outlines of right valve. 1. Male; 2. Gravid female; 3. Copulating female; 4. Medial view, left antennule of male; 5. Medial view, left antenna of male; 6. Dorsal claw of antenna of male; 7-9. Lateral views. 7. Left antenna (proximal portion omitted) of gravid female; 8. Right antenna (proximal portion omitted) of copulating female; 9. Right mandible of male; 10. Medial view, left maxilla (proximal portion omitted) of male; 11. Medial view, right first thoracic leg of male; 12. Lateral view of right copulatory complex of male (part of the clasping apparatus is omitted to show the course of the sperm duct in the basal piece); 13. Clasping apparatus of male. (All figures were prepared with the aid of a camera lucida. Figs. 1-3 are drawn to the scale which accompanies fig. 1; all others, except figs. 6 and 13, are drawn to the scale which accompanies fig. 4.)

Female.—Gravid females are larger than males and copulating females. The overall proportions of the shell (fig. 2) are about the same as they are in the male, but the ventral margin shows a pronounced concavity. The shells of twenty gravid females collected at the type locality on five *P. trowbridgii* ranged in length from about .41 mm to about .47 mm and in height from about .25 mm to about .28 mm.

The shell of the copulating female (fig. 3) is similar to that of the male, although the ventral margin is sometimes slightly concave. The shells of twenty copulating females taken at the type locality on five *P. trowbridgii* ranged in length from about .33 mm to about .40 mm and in height from about .18 mm to about .22 mm.

The antennules of the gravid female resemble those of the male, but the antennae of the two sexes are unlike. The dorsal and middle claws of the antenna of the gravid female (fig. 7) taper gradually from the base like the ventral claw, instead of having the distal half differentiated from the proximal half as is the case in the male. There is a small comb-like structure projecting from the distal podomere outside the basal portion of the ventral claw.

The maxillae and mandibles of the gravid females are like those of the male. The thoracic legs of the female are also similar to those of the male except for the fact that the two setae on the first podomere of the first leg are about the same length, and approximately as long as the longer of the two setae on the first podomere of the male.

As in other species of *Entocythere*, the antennae of copulating females differ from those of gravid females in having only four podomeres (the penultimate podomere being undivided) and two end claws instead of three (fig. 8). The comb-like projection of the distal podomere is lacking in copulating females. Otherwise the appendages of gravid and copulating females are similar.

Taxonomic position.—*Entocythere occidentalis* is rather closely related to *E. riojai* Hoff (1943), *E. equicurva* Hoff (1944), and *E. dobbini* Rioja (1943). The outline of the shell and the general form of the clasping apparatus of the male are similar in these four species. *E. occidentalis* differs from *E. equicurva* in having four teeth instead of two at the distal end of the clasping apparatus. The clasping apparatus of *E. dobbini* typically has two teeth at the distal end, but Rioja (1945) noted four in some individuals. The clasping apparatus of *E. riojai*, according to Hoff, may have three or four teeth at the distal end.

The presence of two teeth on the inner border of the clasping apparatus of *E. occidentalis* is characteristic also of *E. equicurva*, *E. riojai*, and *E. dobbini bicuspide*, a subspecies proposed by Rioja to include individuals which have two teeth instead of three as in the typical form. *E. riojai* and *E. equicurva* occasionally have three teeth instead of two on the inner border. Of the species in this group, *E. occidentalis* and the more typical two-toothed form of *E. equicurva* are the only ones in which the more distal of the two teeth on the inner border of the clasping apparatus is considerably smaller than the more proximal one. The incision at the distal end of the basal piece of the copulatory complex of *E. occidentalis* is a distinctive characteristic of this species.

SUMMARY

A new species of *Entocythere*, *E. occidentalis*, is described from Oregon. This ostracod is commensal on *Pacifastacus leniusculus*, *P. trowbridgii*, and *P. klamathensis* and is found in association with *E. columbia* Dobbin. *E. occidentalis* is closely related to *E. equicurva* Hoff, *E. riojai* Hoff, and *E. dobbini* Rioja.

REFERENCES

BOTT, R. 1950—Die Flusskrebse Europas (Decapoda, Astacidae). Abhandl. senckenberg. naturf. Ges. No. 483.

DOBBIN, CATHERINE N. 1941—Fresh-water Ostracoda from Washington and other western localities. Univ. Wash. Publ. Biol. 4:175-246.

HOFF, C. CLAYTON 1942—The subfamily Entocytherinae, a new subfamily of fresh-water cytherid Ostracoda, with descriptions of two new species of the genus Entocythere. Amer. Midl. Nat. 27:63-73.

———1943—Two new ostracods of the genus Entocythere and records of previously described species. Jour. Wash. Acad. Sci. 33:276-286.

———1944—New American species of the ostracod genus Entocythere. Amer. Midl. Nat. 32:327-357.

RIOJA, ENRIQUE 1943—Estudios carcinológicos. XIV. Nuevos datos acerca de los Entocythere (crus. ostrácodos) de México. An. Inst. Biol. Méx. 14:533-566.

———1945—Estudios carcinológicos. XVIII. Observaciones acerca de las variaciones de la pieza copuladora en Entocythere dobbini, Rioja (crust. ostrácodos). Ibid. 16:419-429.

Nevada Hydrophilidae (Coleoptera)

Ira La Rivers*
University of Nevada, Reno

The present paper is the sixth of a series summarizing the aquatic coleopterous fauna of Nevada. The introductory remarks prefacing the treatment of the Nevada Dytiscidae (La Rivers, 1951) apply to the other groups as well.

The Hydrophilidae, exceeded in number of species among aquatic Coleoptera only by the Dytiscidae, is a dominant element in any body of water containing a varied beetle fauna, and the majority of species are vegetarians. Carnivorous tendencies seem to be generally restricted to the larger forms, and the giant of the family, *Hydrophilus triangularis*, plays a part equally important to that of *Dytiscus* in the economy of most fishponds.

Unlike dytiscids, which are all aquatic, many hydrophilids are terrestrial, one prominent segment of the family being composed entirely of dung inhabitants, and all gradations between the strictly terrestrial and the strictly aquatic types occur, the intermediate species being found along the edges of water.

Swimming efficiency of adult hydrophilids is high, but inferior, generally, to that of dytiscids, since streamlining is slightly less pronounced, and the swimming stroke is a "trot," i.e., an alternate motion, rather than the more competent "gallop" or unison motion of dytiscids. Some hydrophilids seem never to swim, but are crawlers on vegetation, rocks, etc.

The respiratory reservoir in adults is the usual space between elytra and abdominal tergites, and because of the generally greater curvature of the former in this family, is quite large. Whereas the process of renewing air in the reservoir is a simple matter in dytiscids, and quickly executed, hydrophilids have a somewhat roundabout method of accomplishing this. In general, they contact the surface film with the anterior angle of the pronotum, and the club-shaped antennae alternately sweep out of water and back again, on each descent carrying an air bubble entangled in the hairy club. Some of this air is used to maintain the thin hydrostatic film on the venter, the remainder traverses a groove lying between the meso- and metathorax to the air reservoir. A corollary development of these structural modifications is the enlargement of the meso- and metathoracic and the first abdominal spiracles.

Like dytiscids, hydrophilid larvae are nearly all air breathers and must come to the surface periodically to renew their supply, and only an occasional species possesses the ability to extract oxygen directly from the water (viz., *Berosus*, etc.) The tracheae are terminal in position, opening in the bottom of a transverse groove, and are closed by cercal muscles much as in the dytiscids; when the cerci are extended, the tracheal openings close, opening again only upon the flexion of the cerci. Cercal flexure also greatly aids the larva in maintaining its position at the surface by extending the cerci horizontally along the surface film and giving the body support.

* I wish to reiterate my indebtedness to Hugh B. Leech of the California Academy of Sciences for many determinations and much advice on the aquatic Coleoptera. Harry P. Chandler of the California Division of Fish and Game has also been very helpful.

As mentioned, hydrophilids are predominantly an herbivorous family under normal food-supply conditions, but most species turn readily to scavengering and predation with the press of circumstances. The larvae have more native carnivorous tendencies than do the adults—some marked vegetarian species have, in fact, larvae which are normally predaceous. In any survey of larval food habits, particularly with reference to fish management projects, the problem of determining what has been eaten by a given hydrophilid larva is much simpler, usually, than obtaining similar information from dytiscid material, since hydrophilids have macerating instead of suctorial mandibles and it is often possible to retrieve recognizable material from the gut contents for analysis.

KEY TO GENERA OF NEVADA HYDROPHILIDAE—ADULTS

1. First segment of middle and hind tarsi elongate, longer than second 2
First segment very short, shorter than second and often barely visible 3

2(1). Elytra extended below venter; elytral epipleura in great part vertical; metasternal side pieces wide; eyes usually emarginate ..... *Sphaeridium*
Elytra not so extended; epipleura horizontal or wanting; metasternal side pieces moderate or narrow; eyes not emarginate ..... *Cercyon*

3(1). Second segment of hind tarsi short, about equal to third segment; last tarsal segment as long or longer than preceding segments united, or that of the hind tarsi may be shorter ..... *Helophorus*
Second segment elongate, longer than third; last tarsal segment shorter than preceding segments united 4

4(3). Middle and hind tibiae fringed on inner sides with long swimming hairs; pronotum detached in outline ..... *Berosus*
Such tibiae without swimming hairs; pronotum continuous in outline with elytra 5

5(4). Meso- and metasterna raised in a common median keel, produced caudad into a spine 6
Not as above 7

6(5). Last maxillary palpal segment shorter than the preceding segment; length 25 mm or more (*Hydrous*) ..... *Hydrophilus*
Last maxillary palpal segment equal to or longer than preceding segment; length not over 12 mm ..... *Tropisternus*

7(5). Maxillary palpi slender, much longer than antennae, last palpal segment as a rule shorter than penultimate segment (except in *Enochrus* s. str.) (antennae always 9-segmented) (Subtribe Helocharina (Helochareae)) 8
Maxillary palpi stout and short, subequal to antennae, last palpal segment as a rule as long as, or, longer than, the penultimate (last tarsal segment shorter than the preceding ones united) (Subtribe Hydrobiina (Hydrobiae)) 10

8(7). Tarsi 5-4-4 ..... *Cymbiodyta*
Tarsi 5-5-5 9

9(8). Pseudobasal maxillary palpal segment curved, the convexity to the front; mesosternum with a longitudinal lamina (elytra confusedly punctate) ..... *Enochrus*
Pseudobasal segment curved with convexity posteriorly; mesosternum at most feebly protuberant ..... *Helochares*

10(7). Six abdominal sternites visible; elytra without sutural striae (hind trochanters enlarged; hind tibiae curved) ..... *Laccobius*
Only five abdominal sternites visible; elytra with sutural striae 11

11(10). Larger species, longer than 5 mm; elytra striate or with the rows of punctures very pronounced ..... *Hydrobius*
Smaller species, not exceeding 3 mm; elytra confusedly punctate or almost impunctate, never striate; at most, with the punctures subserially arranged ..... *Paracymus*

LARVAE

1. With 9 complete abdominal segments, the 10th reduced but distinct (integument noticeably chitinized; spiracles lateral, well-developed) ..... *Helophorus*
With 8 complete segments, 9-10 reduced and forming a stigmatic atrium (except in *Berosus* in which there is no atrium) 2

2(1). Pseudo-apneustic spiracular system; 7 pairs of very prominent tracheal gills present on abdomen; abdominal segments 9-10 very much reduced and no stigmatic atrium is present ..... *Berosus*
Pseudo-metapneustic spiracular system; tracheal gills not nearly so prominent or absent; abdominal segments 9-10 reduced, forming a stigmatic atrium 3

3(2). Ocular areas round, usually small and more closely aggregated; legs reduced or absent; pleural lobes not prominent; abdomen truncate 4
Ocular areas oval, larger, aggregated but more distant; legs well-developed, visible from above except in *Paracymus;* pleural lobes usually prominent; abdomen not noticeably truncate, more-or-less elongate 5

4(3). Head subspherical; ocular areas aggregated but more distant from each other; mandibles without inner teeth, furrowed on inner side; mentum cordiform; tarsi absent; procercus and appendage of 7th intersegmental membrane fleshy and long ..... *Sphaeridium*
Head ovate; ocular areas closely aggregated and sometimes appearing as one; right mandible with a single inner tooth; mentum short, transverse; legs absent; procercus and appendage of 7th intersegmental membrane reduced and barely indicated ..... *Cercyon*

5(3). First antennal segment distinctly longer than the following two combined; finger-like antennal appendages absent; labro-clypeus with teeth small or absent; mouthparts slender; mandibles grooved internally; stipes not swollen, with setae arranged other than mentioned below; externo-frontal angles of mentum prominent and acute; legs very long; femora with fringes of long swimming hairs; gills present; prostyle present 6
First antennal segment not distinctly longer than the following two together; finger-like antennal appendages present; labro-clypeus with teeth usually well-defined; mouthparts stout; mandibles not grooved internally; stipes large and swollen, usually with an inner row of five stout setae; externo-frontal angles of mentum not prominent, rounded; legs much shorter, femora without fringes of swimming hairs; gills and prostyles absent 7

6(5). Head subspherical: labro-clypeus lacknig teeth; each mandible with a single inner tooth; ligula not longer than first segment of palp; gills absent; pronotum not entirely chitinized ..... *Hydrophilus*
Head subquadrangular, narrowed behind; labro-clypeus with inconspicuous teeth; each mandible with more than one, usually with two, inner teeth; ligula distinctly longer than first palp segment; pronotum entirely chitinized; gills present but more-or-less rudimentary ..... *Tropisternus*

7(5). Frontal sutures parallel and not uniting to form an epicranial suture; left expansion of epistoma much more prominent than the right expansion and with a row of stout setae; ligula absent; reduced sclerites of meso- and metathorax widely separated; tarsi well-developed, about as long as tibiae ..*Laccobius*
Frontal sutures not parallel and may or may not unite to form an epicranial suture; lateral expansions of epistoma similar and usually in line with anterior margin of labro-clypeus, no rows of stout setae except in *Hydrobius;* ligula present and longer than segment 1 of palpus; sclerites of meso- and metathorax reduced but not so widely separated; tarsi less developed, usually much shorter than tibiae 8

8(7). Antennae short and antennal appendages more prominent especially in first-stage larvae; epicranial suture absent; legs reduced; abdomen truncate; cercus with long terminal seta ..... *Paracymus*
Antennae long and antennal appendages less prominent; epicranial suture present but usually short; legs fairly long, not reduced; abdomen narrowed caudally; cercus with a short, terminal seta 9

9(8). Mandibles asymmetrical, the right with two inner teeth, the left with only one; abdomen with prolegs on segments 3-7 ..... *Enochrus*
Mandibles symmetrical, each with 2-3 inner teeth; abdomen lacking prolegs 10

10(9). Labro-clypeus with five distinct teeth, outer left tooth a little distant from the rest; each mandible with three inner teeth; mentum subquadrangular ..... *Hydrobius*

Labro-clypeus with at least six teeth; each mandible with two inner teeth; mentum cordiform 11

11(10). Labro-clypeus with six distinct teeth, placed in two groups, two on left and four on right; mentum covered with small spines; anterior sclerites of metathorax with caudal projection ..... *Helochares*

Labro-clypeus with more than six teeth, those to the right not clearly defined and with several smaller teeth; mentum with small spines only toward base; anterior sclerites of metathorax without caudal projections, rectangular ..... *Cymbiodyta*

HELOPHORINAE

HELOPHORUS Fabricius, 1775

The species of this genus are in a tangled taxonomic condition, and it is not possible at the present time to offer keys to the Nevada species.

H. lecontei Knisch, 1924 (*H. obscurus* Le Conte, 1852).—Nye County (*Darrough Hot Springs*, C. T. Brues, 1930—Hot Spring No. 102 (36.8°C, sp. gr. 1.0025, pH 9.0)); White Pine County (*Monte Neva*, C. T. Brues, 1930—Hot Spring No. 96 (36.3°C, sp. gr. 1.0038, pH 8.4)) (Brues, 1932). I have no specimens.

H. linearis Le Conte, 1855.—Elko County (near *Carlin*, 22(vii)12) (Andrews, 1917). Andrews' specific comment is: "This species was taken with the preceding one [*Rhantus binotatus*] (in the pond west of the bridge near Moline Canyon)." The species was described from one specimen taken at Fort Laramie, Nebraska. I do not have it.

H. lineatus Say, 1823.—Lander County (*Birch Creek Ranch* (the springs here concerned are now known as the Spencer Springs on general state maps), C. T. Brues, 1930—Hot Spring No. 101 (36.2°C, sp. gr. 1.0038, pH 7.3); *Cortez*, C. T. Brues, 1930—Hot Spring No. 103 (40.8°C, sp. gr. 1.0041, pH 8.5) (Brues, 1932); *Watt's Ranch*, C. T. Brues, 1927—Hot Springs No. 23 (30.0°C, sp. gr. 1.0008, pH 8.0), Hot Spring No. 23a (36.0°C, sp. gr. 1.0021, pH 8.5)) (Brues, 1928); Mineral County (*Monte Christo* (SE of Rawhide and not now listed on general state maps), C. T. Brues, 1930—Hot Spring No. 104 (38.3°C, sp. gr. 1.0052, pH 8.7)); Nye County (*Darrough Hot Spring*, C. T. Brues, 1930—Hot Spring No. 102 (36.8°C, sp. gr. 1.0025, pH 9.0)) (Brues, 1932); Washoe County (*Truckee Meadows* (Reno), 5(iv)41, el. 4500 ft. —LaR); White Pine County (*Cherry Creek*, C. T. Brues, 1930—Hot Spring No. 94 (no temperature recorded, sp. gr. 1.0038, pH 8.4), Hot Spring No. 95 (36.3°C, sp. gr. 1.0043, pH 8.4); *Monte Neva*, C. T. Brues, 1930—Hot Spring No. 96 (36.3°C, sp. gr. 1.0038, pH 8.4)) (Brues, 1932). It is probable that all Nevada records of this will eventually prove to be other species (Chamberlain, 1947).

H. linearoides d'Orchymont, 1945.—In a personal communication (1947), the late Mr. K. F. Chamberlain was kind enough to allow me the following information on this species: "I have actually seen specimens from Nevada, but unfortunately these were from a very old collection and the specimens were labelled simply 'NEV.' without precise locality, date or collector's name." I have not seen the species.

HYDROPHILINAE

BEROSINI

BEROSUS Leach, 1817

KEY TO SPECIES

Elytral apices acutely spinose ..... *punctatissimus*

Elytral apices non-spinose, although occasionally prolonged ..... *fraternus*

B. punctatissimus Le Conte, 1852.—Nye County (*Beatty* (Amargosa River), 29(xii)46, el. 3375 ft. —LaR, T. J. Trelease, B. H. Banta & R. G. Miller). This distinctive species has been taken only in southern Nevada. At the collecting locality, in the Amargosa river just south of Beatty, this species was found sparingly in the icy water in weather cold enough to freeze water along the banks each night, associated with the very common *Tropisternus ellipticus* in the ratio of about 5:15, respectively, as well as with rare examples of *Hydroscapha natans*.

B. fraternus Le Conte, 1855.—Washoe County (*Peavine Mountain* (Reno), 9(vi)40, el. 6500 ft. —LaR; *Truckee Meadows* (Reno), 17(x)39, el. 4500 ft. —LaR). Apparently the only species occurring in the vicinity of Reno, it is distinctly larger and darker than the southern Nevada *B. punctatissimus*.

HYDROPHILINI

HYDROPHILUS De Geer, 1774

H. triangularis Say, 1823 (*Hydrous*).—Humboldt County (*6½ mi. S. of Denio*, Oregon) (a town on the state line), C. T. Brues, 1927—Hot Spring No. 16 (39.0°C, sp. gr. 1.0015, pH 8.5) (Brues, 1928); *10½ mi. S. of Denio*, Oregon, C. T. Brues, 1930—Hot Spring No. 118 (36.8°C)) (Brues, 1932); Washoe County (*Truckee Meadows* (Reno), 16(iv)39, el. 4500 ft. —LaR; *Wadsworth Slough*, 20(ix)41, el. 4000 ft. —LaR & T. J. Trelease). The largest aquatic beetle in the state, and very common.

TROPISTERNUS Solier, 1834

KEY TO SPECIES

1. Head, pronotum and elytra margined with yellow ..... *lateralis*
   Pronotum and elytra concolorous 2
2(1). Pseudobasal segment of maxillary palp short, barely or not reaching the eyes, subequal in length to ultimate segment, penultimate segment distinctly shorter ..... *orvus*
   Pseudobasal segment long, reaching to anterior margin of eye, and distinctly longer than ultimate or penultimate segments 3
3(2). Lateral median series of pronotal punctures coarser, 1-2, rarely 4, the anterior ones smaller; body equally obtuse anteriorly and posteriorly, strongly convex in profile ..... *ellipticus*
   Lateral median series of pronotal punctures fine, 4-12 in number, forming an oblique line; body not strongly convex in profile 4
4(3). Pubescent areas of hind femora more extensive though still small, clearly reaching to tip of trochanters; elytra with dual punctation in apical quarter, the large punctures numerous, irregularly spaced, nearly obliterating the small primary punctures ..... *californicus*
   Pubescent areas of metafemora very small, confined to extreme base, barely or not reaching tip of trochanters; elytra nearly uniformly punctate (except for the usual coarse serial punctures) 5
5(4). Hind femora parallel-sided in basal two-thirds, flat, sparsely and moderately coarsely punctate, in large part testaceous; mesosternal keel very finely punctate in both sexes; posterior midtarsal claw of male with the tooth about equidistant from tip-to-base; larger species, less parallel-sided, elytra distinctly greenish ..... *sublaevis*
   Hind femora not parallel-sided in basal two-thirds, the posterior margin gradually curved; metafemora distinctly inflated, more coarsely and numerously punctate, in large part piceous or black; mesosternal keel finely punctate in female, rather sparsely and moderately coarsely punctate in male; posterior mesotarsal claw of male with the tooth larger and more apical; smaller species, more parallel-sided, dorsal color blacker and with a slight bronze luster ..... *columbianus*

T. ellipticus (LeConte), 1855.—Churchill County (*Fallon*, 29(xi)39, el. 3960 ft. —LaR); Humboldt County (*6½ mi. S. of Denio*, Oregon) (a town on the state line), C. T. Brues, 1927—Hot Spring No. 16 (35.8°C, sp. gr. 1.0015, pH 8.5) (Brues, 1928); *Soldier Meadows*[1] (SW corner of the county), C. T. Brues, 1930—Hot Spring No. 113

[1] This is not the true Soldier Meadows, which lies in the bordering hills several hundred feet higher, but, as Dr. Brues states, a locality "about 35 miles almost directly north of Gerlach, Nevada, very close to the road that leads to Soldier Meadows along the western side of the Black Rock Desert." Brues' Hot Spring No. 113 is cooler than the springs of Soldier Meadows, and contains a much less interesting endemic fauna. Although largely unsampled for its arthropod elements, Soldier Meadows proper is, among other things, the type locality for the unique cyprinid fish, *Eremichthys acros* Hubbs & Miller, 1948, part of an interesting relict fish fauna.

(19.2°C, sp. gr. 1.0007, pH 8.8)) (Brues, 1932); Lyon County (*Wabuska Hot Springs,* 20(x)41, el. 4300 ft. —LaR & T. J. Trelease); Nye County (*Beatty* (Amargosa River), 29(xii)46, el. 3375 ft. —LaR, T. J. Trelease, B. H. Banta & R. G. Miller); Washoe County (*Truckee Meadows* (Reno), 18(v)40, el. 4500 ft. —LaR). Very common in Nevada. Originally described from New Mexico and California specimens.

T. sublaevis (Le Conte), 1855.—Esmeralde County (*Fish Lake Valley,* 12(i)40, el. 4500 ft. —LaR); Humboldt County (*Soldier Meadows* (SW corner of county), C. T. Brues, 1930—Hot Spring No. 113 (19.2°C, sp. gr. 1.0007, pH 8.8)); Lander County (*Cortez,* C. T. Brues, 1930—Hot Spring No. 103 (40.8°C, sp. gr. 1.0041, pH 8.5)) (Brues, 1932); Lincoln County (*Hiko,* 17(i)40, el. 3880 ft. —LaR); Nye County (*Darrough Hot Springs,* C. T. Brues, 1930—Hot Spring No. 102 (36.8°C, sp. gr. 1.0025, pH 9.0)); White Pine County (*Monte Neva,* C. T. Brues, 1930—Hot Spring No. 96 (36.3°C, sp. gr. 1.0038, pH 8.4)) (Brues, 1932). A very common species.

(?*T. californica* (Le Conte) 1855).—Eureka County (*Antelope Valley* (SSW of Eureka), C. T. Brues, 1930—Hot Spring No. 100 (35.8°C, sp. gr. 1.0032, pH 9.1)); Humboldt County (*Soldier Meadows* (SW corner of county), C. T. Brues, 1930—Hot Spring No. 113 (19.2°C, sp. gr. 1.0007, pH 8.8)) (Brues, 1932); Lander County (*26 mi. S. of Battle Mountain,* C. T. Brues, 1927—Hot Spring No. 22 (34.5°C, sp. gr. 1.0014, pH 7.8) (Brues, 1928); *Cortez,* C. T. Brues, 1930—Hot Spring No. 103 (40.8°C, sp. gr. 1.0041, pH 8.5)) (Brues, 1932); White Pine County (*Monte Neva,* C. T. Brues, 1930—Hot Spring No. 96 (36.3°C, sp. gr. 1.0038, pH 8.4)) (Brues, 1932).

Previous to Leech's 1946 paper, the name *californicus* was indiscriminately applied to more than one entity. His conclusions regarding the status of true *californicus* are: "According to the specimens before me it is strictly a coastal species, occurring from near Ensenada, Lower California, northward at least to the Winchester Bay region of Oregon." It seems certain now that none of the Great Basin records of *californicus* are tenable, but since *columbianus* and probably *sublaevis* (fide Chandler, 1946) have both been recorded in this area as *californicus,* it is impossible, without re-examination of the specimens involved, to determine which of the many records extant belong to one species or the other. The only recourse at present is to consider such records, for the value they may have, under the original name *californicus,* until further clarification can be attempted. I have found, on recent examination of Reno specimens previously placed as *?californicus* that they seem definitely to be *columbianus,* and it is probable that most Nevada records of *californicus* pertain to *columbianus.*

T. columbianus Brown, 1931.—Nye County (*Pahrump Ranch* (Pahrump Valley), 2(i)47, el. 2500 ft. —LaR, T. J. Trelease & B. H. Banta); Washoe County (*Truckee Meadows* (Reno), 10(x)39, el. 4500 ft. —LaR); White Pine County (*Ely,* 12(ii)40, el. 6470 ft. —LaR). Probably widespread over the state.

T. orvus Leech, 1945.—Described from Mendocino County, California, and recorded with a general range of "northern California, northwestern Oregon, and Washoe Co., Nevada," with "Franktown, Nevada," specifically mentioned. I have no specimens.

T. lateralis lateralis (Fabricius), 1775.—Churchill County (*Sand Springs,* 5(vii)41, el. 4500 ft. —LaR & G. C. Christensen); Washoe County (*Truckee Meadows* (Reno), 10(x)39, el. 4500 ft. —LaR; *Wadsworth Slough,* 20(ix)41, el. 4000 ft. —LaR & T. J. Trelease). A common form. Andrews (1917) recorded *T. lateralis* from Elko County (near *Carlin,* 22(vii)12), taken "in the pond west of the bridge near Moline Canyon," as the synonym *T. limbalis* (Le Conte), 1855.

T. lateralis dorsalis Brulle, 1838.—Churchill County (*Sand Spring,* 5(vii)41, el. 4500 ft. —LaR & G. C. Christensen); Humboldt County (*6½ mi. S. of Denio, Oregon* (a town on the state line), *C. T. Brues,* 1927—Hot Spring No. 16 (35.8°C, sp. gr. 1.0015, pH 8.5); *Paradise,* C. T. Brues, 1927—Hot Spring No. 19 (32.2°, sp. gr. 1.0012, pH 7.5-7.7)); Lander County (*Watt's Ranch,* C. T. Brues, 1927—Hot Spring No. 23 (30.0°C, sp. gr. 1.0008, pH 8.0)); Pershing County (*29 mi. S. of Winnemucca,* C. T. Brues, 1927—Hot Spring No. 20 (36.3°C, sp. gr. 1.0014, pH 8.6)) (Brues, 1928); Washoe County (*Truckee Meadows* (Reno), 10(x)39, 14(vii)40, el. 4500 ft. —LaR).

Whatever the final status of true *T. l. dorsalis,* which, according to Leech (1948), is a well-defined northwestern and mountain subspecies, at least some of the records in the

literature for Nevada are erroneous. For example, at the Sand Spring locality, typical *T. lateralis* and *T. l. dorsalis* occur side-by-side, if the conventional color patterns be taken as indices of the two forms. Since these color patterns have been the criteria in the past, it is certain that designations of *T. l. dorsalis* on this point alone are open to objection here, for the existence of the two phases in the same small pond, with suitable intergradients, shows the presence of masking color variants. Also, as Leech points out, the name *dorsalis* is preoccupied, and he prefers *binotatus* (Walker, 1866) for the form.

HYDROBIINI

HYDROBIINA (Hydrobiae)

HYDROBIUS Leach, 1815

H. fuscipes (Linné), 1758.—Lander County (*Watt's Ranch*, C. T. Brues, 1927—Hot Spring No. 23a (36.0°C, sp. gr. 1.0021, pH 8.5)) (Brues, 1928). I have no specimens.

PARACYMUS Thomson, 1867

P. subcupreus (Say), 1825.—Elko County (*Ruby Valley*, C. T. Brues, 1927—Hot Spring No. 28 (no temperature recorded, sp. gr. 1.0009, pH 9.4)) (Brues, 1928); Eureka County (*Antelope Valley* (SSW of Eureka), C. T. Brues, 1930—Hot Spring No. 100 (35.8°C, sp. gr. 1.0032, pH 9.1)) (Brues, 1932); Humboldt County (*Paradise*, C. T. Brues, 1927—Hot Spring No. 19 (31.8°C, sp. gr. 1.0012, pH 7.5-7.7)) (Brues, 1928); Lander County (*Cortez*, C. T. Brues, 1930—Hot Spring No. 103 (40.8°C, sp. gr. 1.0041, pH 8.5)); Mineral County (*Monte Christo* (near Rawhide), C. T. Brues, 1930—Hot Spring No. 104 (38.3°C, sp. gr. 1.0052, pH 8.7)); Nye County (*Darrough Hot Springs*, C. T. Brues, 1930—Hot Spring No. 102 (36.8°C, sp. gr. 1.0025, pH 9.0); *Duckwater*, C. T. Brues, 1930—Hot Spring No. 99 (29.0°C, sp. gr. 1.0032, pH 8.0); *Sunnyside*, C. T. Brues, 1930—Hot Spring No. 97 (30.8°C, sp. gr. 1.0029, pH 9.3), Hot Spring No. 98 (33.6°C, sp. gr. 1.0042, pH 8.3)) (Brues, 1932). In addition, Dr. Brues recorded specimens from Amedee, California, near the north-central state line, so this species seems to be practically statewide over Nevada.

LACCOBIUS Erichson, 1837

KEY TO SPECIES

Punctures of elytral disc arranged in distinct rows of brownish-colored lines; base of head and pronotum more-or-less distinctly granulated between the punctation; form more briefly oval, shorter and convex; obscure transverse pronotal spot less broken at the sides ..... *agilis*

Punctures of elytral disc more irregular, confused, not definitely aligned in a colored series; form more elongated or elliptical, less convex; obscure pronotal spot more broken laterally, less transverse ..... *ellipticus*

L. agilis Randall, 1838.—Elko County (*Ruby Valley*, C. T. Brues, 1927—Hot Spring No. 28 (38.0°C, sp. gr. 1.0009, pH 9.4); *Wells*, C. T. Brues, 1927—Hot Spring No. 27 (40.0°C, sp. gr. 10017, pH 7.1)); Eureka County (*7 mi. S. of Beowawe*, C. T. Brues, 1927—Hot Spring No. 24 (38.8°C, sp. gr. 1.0020, pH 9.6)) (Brues, 1928); Humboldt County (*Soldier Meadows* (35 mi. NE of Gerlach), C. T. Brues, 1930—Hot Spring No. 113 (19.2°C, sp. gr. 1.0007, pH 8.8)) (Brues, 1932); Lander County (*26 mi. S. of Battle Mountain*, C. T. Brues, 1927—Hot Spring No. 22 (34.5°C, sp. gr. 1.0014, pH 7.8) (Brues, 1928); *Birch Creek Ranch* (springs concerned are known now as Spencer Springs on state maps), C. T. Brues, 1930—Hot Spring No. 101 (36.2°C, sp. gr. 1.0038, pH 7.3); *Cortez*, C. T. Brues, 1930—Hot Spring No. 103 (40.8°C, sp. gr. 1.0041, pH 8.5)); Nye County (*Beatty* (Amargosa River), 29(xii)46, el. 3375 ft. —LaR, T. J. Trelease, B. H. Banta & R. G. Miller; *Sunnyside*, C. T. Brues, 1930—Hot Spring No. 98 (33.6°C, sp. gr. 1.0042, pH 8.3)) (Brues, 1932); White Pine County (*Monte Neva*, C. T. Brues, 1930—Hot Spring No. 96 (36.3°C, sp. gr. 1.0038, pH 8.4)) (Brues, 1932).

L. ellipticus Le Conte, 1855.—Eureka County (*Cortez Range* (north end), 9(vii)12) (Andrews, 1917); Washoe County (*Pyramid Lake*, C. T. Brues, 1930—Hot Spring No. 105 (29.8°C, sp. gr. 1.0064, pH 8.0), Hot Spring No. 106a (29.0°C, sp. gr. not taken, pH 8.4)) (Brues, 1932). The Cortez Range record presumably represents a Eureka

County locality, although the University of Michigan party worked out of Carlin, Elko County. I have no specimens.

"*Laccobius* sp.—several specimens taken with the preceding species [*L. ellipticus*] are not referrable to it nor to others of the genus" is a notation occurring in Andrews, 1917.

HELOCHARINA (Helochareae)

HELOCHARES Mulsant, 1844

H. normatus Le Conte, 1861 (also *Chasmogenus*).—Nye County (*Beatty* (Amargosa River), 29(xii)46, el. 3375 ft. —LaR, T. J. Trelease, B. H. Banta & R. G. Miller). This is the first Nevada record for the species, which is well-known to the area immediately south of the state.

ENOCHRUS Thomson, 1859

KEY TO SPECIES

1. Last two maxillary palpal segments subequal (Subgenus *Enochrus*) ..... *carinatus*
   Last maxillary palpal segment shorter than the third segment 2

2(1). Fifth ventral abdominal segment with a semicircular notch fringed with stiff russet or golden hairs; sometimes the notch is shallow and poorly indicated, but in that case, the fringe of hairs, distinct from the surrounding pubescence, is present (Subgenus *Methydrus* Rey, 1885) ..... *pygmaeus*
   Fifth ventral segment without a ciliated notch, the posterior margin entire, without a fringe of stiff hairs at middle (Subgenus *Lumetus* Zaitzev, 1908) 3

3(2). Prothoracic dorsum entirely piceous ..... *conjunctus*
   Prothoracic dorsum entirely testaceous ..... *diffusus*
   Prothoracic dorsum largely piceous, paler at margins ..... *hamiltoni*

E. carinatus (Le Conte), 1855 (also *Philhydrus*).—Clark County (*Hidden Ranch* (Pahrump Valley), 3(i)47, el. 2500 ft. —LaR, T. J. Trelease, & B. H. Banta); Nye County (*Darrough Hot Springs*, C. T. Brues, 1930—Hot Spring No. 102 (36.8°C, sp. gr. 1.0024, pH 9.0) (Brues, 1932); *Manse Ranch* (Pahrump Valley), 3(i)47, el. 2500 ft. —LaR, T. J. Trelease & B. H. Banta); Washoe County (*Pyramid Lake Pinnacles* (erroneously listed as a California locality in the original), C. T. Brues, 1930—Hot Spring No. 106a (29.0°C, sp. gr. 1.0064, pH 8.4), Hot Spring No. 107 (36.5°C, sp. gr. 1.0074, pH 8.4) (Brues, 1932); *north end Pyramid Lake* (in springs back from shore), 16(viii)41, el. 3900 ft. —LaR, T. J. Trelease & G. C. Christensen).

At the Hidden Ranch locality, *E. carinatus* was found clinging to the sides and bottoms of stones in water at the edges of the small stream which, at intervals, had a solid crust of ice over it. Associated species were *Cymbiodyta dorsalis* and *Deronectes striatellus*.

E. diffusus (Le Conte), 1855 (also *Philhydrus*).—Nye County (*Beatty* (Amargosa River), 29(xii)46, el. 3375 ft. —LaR, T. J. Trelease, B. H. Banta & R. G. Miller). My first record for the state. Associated species here included *Tropisternus ellipticus, Deronectes striatellus, Peltodytes simplex, Berosus punctatissimus, Laccophilus decipiens, Helochares normatus, Enochrus pygmaeus, Laccobius agilis* and *Hydroscapha natans*, all living in rather swift waters of the tiny Amargosa, which froze at the banks each night.

E. conjunctus (Fall), 1901 (also *Philhydrus*).—Lander County (*Cortez*, C. T. Brues, 1930—Hot Spring No. 103 (40.8°C, sp. gr. 1.0041, pH 8.5)); Nye County (*Darrough Hot Springs*, C. T. Brues, 1930—Hot Spring No. 102 (36.8°C, sp. gr. 1.0024, pH 9.0)); Washoe County (*Gerlach*, C. T. Brues, 1930—Hot Spring No. 108 (32.8°C, sp. gr. 1.0061, pH 7.5)) (Brues, 1932). Originally described from Lake Tahoe. I have no specimens.

E. pygmaeus (Fabricius), 1792 (*E. nebulosus* (Say), 1824) (also *Philhydrus*).—Elko County (*Ruby Valley*, C. T. Brues, 1927—Hot Spring No. 28 (35.6°C, sp. gr. 1.0009, pH 9.4); *Wells*, C. T. Brues, 1927—Hot Spring No. 27 (40.0°C, sp. gr. 1.0017, pH 7.1)) (Brues, 1928); Eureka County (*Antelope Valley* (SSW of Eureka), C. T. Brues, 1930—Hot Spring No. 100 (39.2°C, sp. gr. 1.0032, pH 9.1) (Brues, 1932); *7 mi. S. of Beowawe*, C. T. Brues, 1927—Hot Spring No. 24 (40.5°C, sp. gr. 1.0020, pH 9.6)); Humboldt County (*25 mi. S. of Denio, Oregon* (a town on the state line), C. T. Brues, 1927—Hot Spring No. 15 (35.2°C, sp. gr. 1.0000, pH 8.4); *Paradise*, C. T. Brues, 1927—Hot Spring No. 19 (31.8°C, sp. gr. 1.0012, pH 7.5-7.7)) (Brues, 1928);

Lander County (*Cortez*, C. T. Brues, 1930—Hot Spring No. 103 (40.8°C, sp. gr. 1.0041, pH 8.5) (Brues, 1932); *Watt's Ranch*, C. T. Brues, 1927—Hot Spring No. 23a (36.0°C, sp. gr. 1.0021, pH 8.5)) (Brues, 1928); Nye County (*Beatty* (Amargosa River), 29(xii)46, el. 3375 ft. —LaR, T. J. Trelease, B. H. Banta & R. G. Miller; *Duckwater*, C. T. Brues, 1930—Hot Spring No. 99 (29.0°C, sp. gr. 1.0032, pH 8.0) (Brues, 1932); *Pahrump Ranch* (Pahrump Valley), 2(i)47, el. 2500 ft. —LaR, T. J. Trelease & B. H. Banta); Pershing County (*29 mi. S. of Winnemucca*, C. T. Brues, 1927—Hot Spring No. 20 (38.0°C, sp. gr. 1.0014, pH 8.6)); Washoe County (*Steamboat* (near Reno), C. T. Brues, 1927—Hot Spring No. 8 (33.0°C, sp. gr. 1.0030, pH 8.0)) (Brues, 1928). The Pahrump locality is the southernmost record for the species in Nevada.

E. hamiltoni (Horn), 1890 (also *Philhydrus*).—Elko County (*Ruby Valley*, C. T. Brues, 1927—Hot Spring No. 28 (40.0-41.5°C, sp. gr. 1.0009, pH 9.4)) (Brues, 1928). Dr. Brues also took the species at two localities in California near the Nevada line, so it is evident that it has a wide distribution in the state. It was originally described from a series representing several localities from coast-to-coast, the western U. S. specimen listed as being from "northern California and Oregon."[2] I have no specimens.

According to Horn (1890a), *E. cuspidatus* (Le Conte), 1878, "occurs in the mountain regions of California near Lake Tahoe," and as such is likely to be found in adjacent Nevada. It belongs to the subgenus *Enochrus* as outlined in the key to species above, and can be distinguished from *E. carinatus* by its lack of the prosternal carina from which *carinatus* derives its name.

CYMBIODYTA Bedel, 1880

KEY TO SPECIES

1. Five outer elytral striae entire (color black with pale border) ..... *dorsalis*
   Only four outer elytral striae entire 2
2(1). Overall color black, with a pale border; coarser punctures of alternate elytral intervals 3 to 9 well-impressed and conspicuous ..... *morata*
   Elytra brownish, thorax with a large black spot reaching both apex and base, and head black; coarser punctures of alternate elytral intervals 3 to 9 feebly impressed and scarcely distinguishable ..... *imbellis*

C. dorsalis (Motschulsky), 1859—Clark County (*Hidden Ranch* (Pahrump Valley), 3(i)47, el. 2500 ft. —LaR, T. J. Trelease & B. H. Banta); Humboldt County (*Soldier Meadows* (35 mi. NE of Gerlach, in extreme SW Humboldt County), C. T. Brues, 1930—Hot Spring No. 113 (19.2°C, sp. gr. 1.0007, pH 8.8)) (Brues, 1932); *Paradise Valley dunes* (8 mi. N of Winnemucca), 18(vi)41, el. 4800 ft. —LaR & G. C. Christensen); Washoe County (*Truckee Meadows* (Reno), 8(ii)41, el. 4500 ft. —LaR); White Pine County (*Ely*, 12(ii)40, el. 6470 ft. —LaR). Commonly distributed over the state. See *Enochrus carinatus*.

C. imbellis (Le Conte), 1861.—Elko County (*Carlin*, 29(vii)12) (Andrews, 1917). Listed by Andrews as taken "on the Carlin Peaks," probably at an elevation approximating 6,000 feet. This is one of the small group of aquatic records (among others) added to the Nevada literature by the University of Michigan's Walker—Newcomb expedition to northeastern Nevada in 1912. I do not have the species.

C. morata Horn, 1890.—Washoe County (*White Rock Spring*, Pah Rah Mountains at SW edge of Pyramid Lake, 8(iv)51, el. 5600 ft. —LaR & G. C. Christensen). Described from New Mexico specimens.

SPHAERIDIINAE

SPHAERIDIUM Fabricius, 1775

S. scarabaeoides (Linné), 1758.—Elko County (*Elko*, 17(vi)39, el. 5000 ft. —LaR); Humboldt County (*Paradise-National Summit*, 21(vi)41, el. 7600 ft. —LaR & G. C.

---

2 Leech (1950): ". . . true *hamiltoni* is a brackish-water species, rarely occurring at any distance inland. I have seen it from the coasts of New Hampshire, New York, New Jersey, Maryland, and Florida. . . . On the Pacific Coast it occurs in similar habitats, but as two recognizable subspecies."

Christensen); Washoe County (*Truckee Meadows* (Reno), 9(iii)41, el. 4500 ft. —LaR). This European species is now a common sight in most parts of the United States, and is present in Nevada wherever suitable animal manures are found, associated with the histerids, staphylinids and aphodiines common to such situations.

CERCYON Leach, 1817

KEY TO SPECIES

1. Elytral striae replaced by rows of punctures; (body very convex) ..... *tristis*
   Elytral striae impressed, at least in part of their length 2
2(1). Mesosternal elevation oval, only twice as long as wide (upper surface alutaceous) ..... *nevadanus* (*kulzeri*)
   Mesosternal elevation linear or narrowly lanceolate 3
3(2). Lateral marginal prothoracic line extended at least slightly along the basal margin (length 1.5-2.0 mm, yellow elytra) ..... *quisquilius*
   Lateral marginal prothoracic line reaching the hind angles only (elytra alutaceous) ..... *opacellus*

C. opacellus Fall, 1924 (*C. lugubris* Paykull, 1798 of Horn, 1890, nec true *C. lugubris* of Paykull). My only available record for this is as *C. lugubris* of Horn (1890), who wrote:

"Specimens in my cabinet from Nevada have the striae of the disc somewhat more distinct, and consequently more distinctly punctate than those from Canada or others from Sweden, but I can find no reason for separating them as a distinct species."

Fall (1924) gave the new name *opacellus* to what Horn called the *lugubris* of Paykull, feeling that the two were not the same species; in so doing, however, he erroneously restricted *opacellus* to the eastern United States, and used this distinction of distribution in a key to the species. In the same paper Fall described *californicus*, which seems evident now, from Blackwelder (1931), to be a synonym of Knisch's (1922) *kulzeri*, also described from California, and which Fall was not aware of. Blackwelder (1931) repeated Horn's Nevada record.

C. nevadanus Knisch, 1924(a). This was described from three of Morrison's specimens in the Stockholm museum, labelled simply "Nevada." Knisch identified it closely with *kulzeri*, to which it will run in the above key. No specimens are available, and I cannot at present determine its relationship to *kulzeri*. However, because the original description is difficult to obtain, and may be of future aid to others in locating the species, I append it below:

"*Cercyon* (*s.* str.) *nevadanus* Knisch nov. spec.

"Klein, langlich oval, nicht sehr hoch gewölbt, rückwärts etwas zugespitzt. Oberseite schwarz, die Seiten des Halsschildes, die Spitzen der Flügeldecken mit Ausnahme der dunklen Naht, und zum Teil auch deren Seitenrand verwaschen braunrot, matt. Die Unterseite schwarz, die Kiefertaster dunkelbraun oder schwarz, die Fühler braungelb mit dunklerer Keule, die Beine rotbraun.

"Die ganze Oberseite im Grunde fein schagriniert. Kopf und Halsschild fein aber deutlich punktierten Punktreihen, deren Punkte in den seitilchen Reihen und gegen die dem vierten Streifen der Flügeldecken gegenüberliegenden Grübchen.

"Die Flügeldecken noch matter als Kopf und Halsschild, mit zehn mässig vertieften, deutlich punktierten Punktreihen, deren Punke in den seitilchen Reihen und gegen die Spitze hin allmählig etwas kräftiger werden, woselbst sie aber weniger scharf eingekerbt sind. Der zehnte, nächst dem Seitenrande gelegene Streifen ist durch eine nur in der Basalhälfte ausgebildete, aus ziemlich distant angeordneten Punkten bestehende Punctreihe ersetzt. An der Spitze sind die Punkte sämtlicher Streifen wessentlich spärlicher aber kräftiger. Die Zeischenraume sind durchaus flach, auf mattem Grunde äusserstfein, nur microskopisch erkennbar und weitläufig punctiert.

"Die Fläche der Mesosternallamelle kaum doppelt so lang als breit, kaum zugespitzt, auf undeutlich schagriniertem Grunde gut erkennbar und nicht sehr dicht punktiert. Die Medianpartie des Metasternums im grunde deutlich schagriniert, fein aber deutlich und etwasweitläufig punktiert, fettglänzend. Schenkellinien nicht nach vorne verlängert.

"Long. 1.8-2 *mm*.

Patria: Nevada (Morrison). Drei Exemplare aus dem Reichsmuseum in Stockholm.

"Die Art steht durch die Schagrinierung der ganzen Oberseite dem *Cercyon Kulzeri* m. sehr nahe, ist aber durchschnittlich grösser, und unterscheidet sich auch sonst durch mattere Oberseite, weniger helle Apikalfärbung der Flügeldecken, sowie viel feinere, nur mikroskopisch erkennbare Punktirung der Intervalle derselben, und ist ausserdem durch die Schagrinierung der Fläche der Mesosternallamelle und der Medianpartie des Metasternums hindlänglich verschieden."

C. quisquilius (Linné), 1761.—Humboldt County (*Paradise-National Summit* (Santa Rosa Mts.), 21(vi)41, el. 76000 ft. —LaR & G. C. Christensen); Washoe County (*Truckee Meadows* (Reno), 2(iii)41, el. 4500 ft. —LaR). This European species is now commonly distributed over the United States, and is found throughout Nevada in cattle manure, associated with *Sphaeridium scarabaeoides, Aphodius fimetarius*, etc.

C. tristis (Illiger), 1801. Horn's 1890, 1890b listing is: "Specimens are known to me from Ohio and Michigan, westward to Iowa, Dakota, California, Washington and Nevada. In the eastern hemisphere it occurs from northern Europe to Siberia." Blackwelder (1931) re-enters the Horn record. I have no specimens.

REFERENCES

ANDREWS, A. W. 1917—Coleoptera collected in northeastern Nevada by the Walker-Newcomb expedition of the University of Michigan. Univ. Mich. Mus. Zoöl. Occas. Paper 48:1-3.

BLACKWELDER, R. E. 1931—The Sphaeridiinae of the Pacific Coast (Coleoptera, Hydrophilidae). Pan-Pac. Ent. 8(1):19-32.

BRUES, C. T. 1928—Studies on the fauna of hot springs in the western United States and the biology of thermophilous animals. Amer. Acad. Arts & Sci. Proc. 63(4):139-228.

———1932—Further studies of the fauna of North American hot springs. Amer. Acad. Arts & Sci. 67(7):186-303.

CHAMBERLAIN, K. E. 1947—Personal communication.

CHANDLER, H. P. 1946—Personal communication.

FALL, H. C. 1924—Some notes on *Cercyon*, with descriptions of three new species. Psyche 31(6):247-253.

HORN, G. H. 1890a—Notes on some Hydrobiini of boreal America. Amer. Ent. Soc. Trans. 17:237-278.

———1890b—A revision of the Sphaeridiini inhabiting boreal America. Ibid. 17:279-314.

HUBBS, C. L. AND R. R. MILLER 1948—Two new, relict genera of cyprinid fishes from Nevada. Univ. Mich. Mus. Zoöl. Occas. Paper 507:1-30.

KNISCH, A. 1924a—Zwei neue Nearktische Palpicornier (Co. *Hydrophilidae* Op. 13). Entomologischer Anzeiger 4(5):55-56.

LA RIVERS, I. 1951—Nevada Dytiscidae (Coleoptera). Amer. Midl. Nat. 45(2):392-406.

LEECH, H. B. 1946—Remarks on some Pacific Coast species of *Tropisternus* (Coleoptera). Canad. Ent. 77(10):179-184.

———1948—Contributions toward a knowledge of the insect fauna of Lower California. No. 11. Coleoptera: Haliplidae, Dytiscidae, Gyrinidae, Hydrophilidae, Limnebiidae. Calif. Acad. Sci. Proc. (4th series) 24(11):375-484.

———1950—New species and subspecies of nearctic water beetles (Coleoptera: Dytiscidae and Hydrophilidae). Wasmann Collector 7(6):243-256.

Ecology of Simuliid Vectors of Onchocerciasis in Guatemala[1,2,3]

Herbert T. Dalmat

National Institutes of Health, Public Health Service, Bethesda, Md. and Pan American Sanitary Bureau

Onchocerca volvulus, the parasite causing human onchocerciasis, was first described from Africa by Leuckart in 1893. Although early workers speculated a good deal concerning the transmission of the parasite from one person to another, it was the discovery of the presence of the disease in Guatemala by Robles in 1915 (Calderón, 1917; Robles, 1919) that actually stimulated extensive investigations of onchocerciasis and its transmission. The disease is now known to exist in Africa, Guatemala, Mexico, and Venezuela.

Robles (1919) after discovering the presence of onchocerciasis in Guatemala, was inclined to believe the vector was a day-biting insect since people who lived outside of the disease zone, and entered it only during the day to work on the coffee plantations, became infected. He then voiced his suspicion that two anthropophilic species of *Simulium* flies found in the endemic region were probably the vectors, but he did not carry out investigations to verify his belief. It remained for Blacklock (1926), working in Africa, to demonstrate that *Simulium* spp, might be the vectors. He was able to infect wild-caught *Simulium damnosum* on infected patients and to trace the subsequent development of the parasite in the gut, thorax, head and proboscis of the flies. Infective larvae were found in the proboscis as early as 7 days after the infective meal. Sharp (1927) and Bequaert (1929), working in Africa, Hoffmann (1930a, b, c, d, e; 1931), Vargas (1948), and Vargas and Ruiz Reyes (1949) in Mexico, and Strong (1931a, b, c; 1934) and De León (1940a, b) in Guatemala also studied the development of microfilariae in *Simulium* spp. Wanson, Henrard, and Peel (1946), working in the Belgian Congo, were the first to report the entire development of *O. volvulus* in laboratory-reared *S. damnosum*. Like Blacklock, they found that the microfilariae reached the infective stage in only 6 to 7 days. In Guatemala, attempts to repeat the experiments of those investigators with local *Simulium* species have not met with equal success since it has not been possible to induce laboratory-reared flies to take blood meals. Gibson and Dalmat (1952) found a high percentage of wild-caught *S. exiguum* naturally infected with "sausage" forms of what they believe may be *Onchocerca gutturosa* of cattle. Gibson (1951a), using wild-caught

1 This report is part of a study jointly supported by the Laboratory of Tropical Diseases of the National Institutes of Health, Public Health Service and the Pan American Sanitary Bureau in cooperation with the Dirección General de Sanidad Pública of the Republic of Guatemala. The project was aided by a reseach grant from the National Institutes of Health, Bethesda, Maryland.

2 The author wishes to express his thanks to Mr. Claudio Urrutia Evans, Director of the National Observatory of Guatemala, for his assistance in securing meteorological and geographical data concerning Guatemala, and to Mr. Arden O. Lea, Jr. for his review of the manuscript and numerous helpful suggestions.

3 Presented as part of the symposium on "Ecology of Vectors of Parasitic Diseases" at the annual meetings of the American Society of Parasitologists, Chicago, November 15-17, 1951.

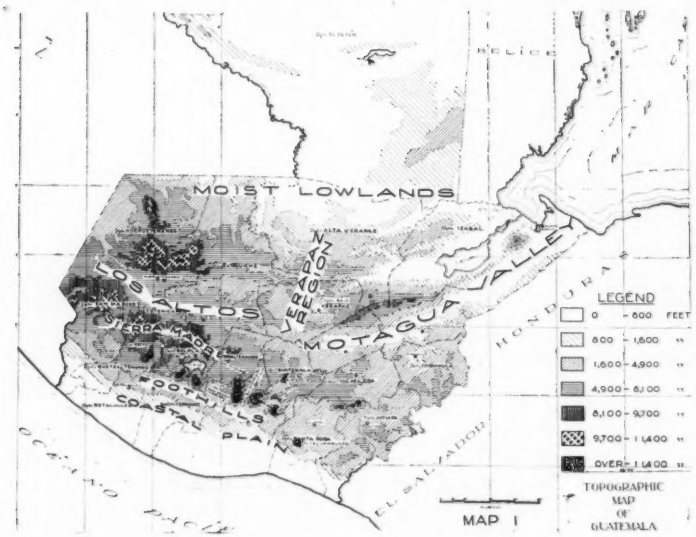
flies, traced the development of *O. volvulus* in *S. metallicum* up to 166 hours; in *S. callidum* up to 176 hours; and in *S. ochraceum* up to 48 hours after the flies had taken an infective blood meal.

The aforementioned investigations indicate that *Similium* spp. are probably the vectors of onchocerciasis. The finding of *Simulium* spp. naturally infected with microfilariae of *O. volvulus*, the successful infection of flies, with subsequent development of the microfilariae, plus epidemiological evidence concerning the distribution of the anthropophilic species in Guatemala, quite definitely incriminate the Simuliidae as the vectors of the disease.

The present ecological study of the principal anthropophilic species of Simuliidae of Guatemala was made during a 4-year period, 1947-1951, as part of a more complete investigation of this family in relation to its role in the transmission of onchocerciasis. The center of investigations was established in the Municipalidad of San Pedro Yepocapa, Department of Chimaltenango, a highly endemic region of the disease. This area is more fully discussed below.

GEOGRAPHY AND CLIMATE OF GUATEMALA

It has often been said that Guatemala has more variation in altitude in proportion to surface area, as well as greater diversity of climate, than any other portion of the earth. Progressively from the Pacific Ocean, certain geographical areas can be distinguished: Bordering on the ocean, the coastal plain appears as a 30-mile wide band which rises uniformly to an elevation of 1000-2000 feet. From this the foothills of the Sierra Madre continue to rise more rapidly and irregularly to an altitude of 5000-6000 feet, and these are followed by the volcanic mountains (Sierra Madre) which rise to heights approaching 14,000 feet. There then follow the plateau region (Los Altos); the Vera Paz region with its low mountains and luxuriant rain forest, abundant streams, and almost daily rains; the semi-arid valley of the Motagua River; and finally the



moist lowlands of the Atlantic Coast, including the Plain of the Petén (map 1).

The great variations in temperature arise between zones of different altitudes, while in any one area the average daily temperature varies but little throughout the year. The temperature zones have been termed "tierra caliente" (hot region) for the coastal plain (sea level to 2000 feet); "tierra templada" (temperate region) for the foothills (from 2000 to 6500 feet of altitude); and "tierra fría" (cold region) for the mountains which extend upwards of 6500 feet. In any particular region of Guatemala, temperature may be considered to be of lesser importance than humidity in its effect on the development of insects. Since the fluctuation from the mean daily temperature is slight in any 24-hour period, and the mean daily temperature is relatively constant throughout the year, insect development can continue at approximately a uniform rate. Thus it must be that the amount and extreme seasonal fluctuations of the rains must exert the greater effect on the development of insects within a region. Startling and abrupt variations in climatic conditions may be found in any one zone, due to the particular location of the area, the direction of the slope, or degree of exposure to prevailing winds.

ONCHOCERCIASIS ZONE

The principal onchocerciasis zone is situated in the foothills region, extending as a 70-mile-long band along the Pacific slope of the Sierra Madre. The volcanoes comprising this range rise out of the coastal plain like a solid wall and run in a general direction from southeast to west, dividing the country more or less into two parts. In the onchocerciasis zone there is a pronounced dry and wet season, the latter being caused by the southwest winds from the Pacific which blow somewhat irregularly from May to November. The high volcanoes are the centers of little areas of precipitation and these have their effect on the surrounding country. With the western slopes all facing the Pacific and the eastern slopes, at the higher altitudes, intercepting the northeast trade-winds, there is almost daily precipitation somewhere on their summits.

DESCRIPTION OF STUDY AREA

The major part of the investigations here reported was made in the Municipalidad of Yepocapa, Department of Chimaltenango, an area of approximately 50 square miles, extending from 1600 to over 5500 feet above sea level. Our central field laboratory is located in the town of Yepocapa itself.

There are 2200 inhabitants in the town. Including the people living on and employed by the coffee fincas (plantations) within the entire Municipalidad of Yepocapa, there are 8200 inhabitants, of whom 74 per cent are Mayan Indians. The town is situated on the western slopes of the volcanoes Acatenango and Fuego at an altitude of 4850 feet. The precipitation, temperature, and winds are all affected by this rather unique location which permits full exposure to the Pacific, along with exposure to more local conditions effectuated by the overlooking eastern valcanoes. A 3-year summary of the weather of the Yepocapa region is as follows: Mean annual temperature 70.3°F, with mean annual maximum and minimum temperatures of 79.2° and 61.3°F respectively; mean monthly maximum temperature highest in May (82°F) and mean monthly minimum temperature lowest in January (58.6°F); aver-

age annual precipitation, 121 inches; winds usually variable during the day, but strongest from November through February.

In a comparison of the meteorological data of the Yepocapa region with those of other endemic areas, almost all conditions seem to be similar. However, it was noted that the difference between the average annual maximum temperature and the average annual minimum temperature becomes smaller as the altitude increases. It may be that the more extreme temperatures found at altitudes above and below the onchocerciasis zone are factors that contribute to the absence of the disease in such regions by limiting either the breeding potential of important vector species or the development of microfilariae. In altitudes above the onchocerciasis zone, the absence of the disease may be the result of the low temperature of the water, which inhibits the larval development of certain of the anthropophilic species. However, there are areas outside of the infected zones that do have the same general meteorological characteristics as areas within the endemic zones. Thus, it appears that temperature and humidity are not in themselves sufficient to determine the presence or absence of the disease.

CLASSIFICATION OF STREAM-BREEDING PLACES AS RELATED TO ECOLOGY

The types of streams comprise another environmental factor of importance in the breeding of *Simulium* species. These can be classified as follows: (a) infant; (b) young; (c) adolescent; (d) mature; and (e) old.

An *infant* stream is one formed by the convergence of several minute trickles of water, generally originating as collections of underground or cliff seepage. This stream may vary from one inch in width to about one foot. It has no definite wall or cross-section and the water channel seems almost haphazard. The stream bed is not very different from the contiguous dry areas. Vegetation, rather than being of a truly aquatic type, appears to be composed of trailing parts of plants that grow along the sides of the water course, as well as of debris and decaying leaves that also cover the adjacent ground. Such streams, which may enter and emerge from the ground several times as they pass along a slope, are often tributaries of young or adolescent streams. Only anthropophilic species are found breeding in infant streams.

The *young* stream is relatively narrow, with steep walls, and V-shaped cross-sections. It has few, if any, tributaries and these are very short. The stream presents a zigzag, ungraded pattern, often with rapids and small falls, and sometimes pools. Characteristically it has abundant emergent and cover vegetation and small deposits of debris. The bed of the young stream consists mainly of an arenaceous mixture topped with small to large stones, and, very seldom, with large rocks. In some streams the sand accumulates around the large rocks to such a height that the upper faces of the rocks themselves form the main part of the stream bed, the spaces between them being filled with sand and gravel. The walls of the stream may be composed of earth, vegetation, rocks, or any combination of these. These streams serve as breeding places for the anthropophilic species primarily.

The *adolescent* stream has the walls less steep than the young one, the falls and rapids usually are replaced by a more graded river bed, the zigzag pattern gives way to meanders and the river assumes a dendritic pattern rather than a singular one. The bed is approximately like that of the young stream, still

supporting a large growth of emergent vegetation and being shaded by dense growth. Both anthropophilic and zoophilic species of *Simulium* breed in these streams.

The more *mature* stream exhibits a broad U-shaped profile. There is usually a complex network of streams with piracy as a common manifestation. Because of the extensive number of tributaries, a large part of the adjoining region is brought more to the slope of the river bed. Deposition may occur, forming narrow flood plains, sandbars, and beaches. This type of stream favors the breeding of zoophilic species of *Simulium* although good numbers of anthropophilic species will also be found in them.

The *old* river flows through a terrain which exhibits a very gentle rolling topography with a minimum vertical slope. There is a great reduction in the number of tributaries with the single stream remaining dominant. In spite of the small grade, the flow is great except toward the mouth of the river where it usually becomes sluggish. Wide beaches, often much wider than the main stream itself, prevail along many sections of such a river. There is practically no emergent vegetation and the shrub and tree line is far removed from the water's edge. Small, smooth, somewhat rounded boulders are often dispersed along the shore line with driftwood deposited amongst them. Almost without exception these streams support only the development of zoophilic species.

The permanent streams and rivers of the Yepocapa onchocerciasis zone are generally of the *infant*, *young* or *adolescent* type. The formation of the *infant* stream has already been discussed. The *young* and *adolescent* streams are formed from an underground spring or from several such springs that unite to form a single stream of water. This leaves the ground at a point where the natural curvature of the land reaches the water table level and where the earth is sufficiently porous. It then joins other similar currents to form a resultant larger stream. During the dry season of the year (November to April) the volume of the permanent streams and rivers remains practically constant; in the wet season (May to October) the volume is greatly increased by flash swells during, and immediately following a heavy rain. These subside again as soon as the surface run-offs have been channelled away. The composition of a stream bed, to some extent, determines what age a particular locality in the stream will have, and this, in turn, is a controlling factor in the breeding of various species of *Simulium*. As evidence of this fact, where bare sand or gravel alone form the bed, there will be no breeding; many zoophilic species prefer the flat faces of the larger rocks for anchorage and they are therefore not often found in the younger streams which are lacking in such substrata; a few of the zoophilic species prefer mud; the anthropophilic species, in general, prefer emergent vegetation and debris found in young streams.

PREDATORS

A survey was made of those animals that may serve as predators of the larvae and adults of *Simulium* spp. in the Yepocapa onchocerciasis region. Among the groups collected were annelids, crustaceans, insects, frogs, fish, and birds. The following represent the most important predators found, as evidenced by the presence of large numbers of simuliid larvae and adults, or parts of their exoskeleton, in the intestinal contents:

Name of Predator	Form of Blackfly Found in Digestive Tract
Corydalus sp. (Dobsonfly larvae)	Larvae
Libellula sp. (Dragonfly nymphs)	Larvae
Hetaerina sp. (Damselfly nymphs)	Larvae
Smicridea sp. (Caddis worms)	Larvae
Abedus ovatus (Electric-light bug nymphs)	Larvae and Adults
Profundulus punctatus (Minnows)	Larvae and Adults
Amazilia candida pacifica (Hummingbird)	Adults
Myiodynastes luteiventris luteiventris (Southern sulphur-bellied flycatcher)	Adults
Onychorhynchus mexicanus mexicanus (Mexican flycatcher)	Adults
Cissilopha melanocyanea melanocyanea (Hartlaub's jay)	Adults
Vermivora peregrina (Tennessee warbler)	Adults
Basileuterus culicivorus culicivorus (Lichtenstein's warbler)	Adults
Vireo solitarius solitarius (Blue headed vireo)	Adults

The numbers of blackfly larvae and adults found in insects and birds were so small that it is doubtful whether these predators play an important role in reducing the simuliid population. On the other hand, examination of over 200 minnows yielded numerous larvae in all but 10, these being fry too small to feed on insects as large as the blackflies. In addition, the contents of the digestive tract of 12 of these fish contained parts of adult flies, indicating that the fish also devour the simuliids as the latter are emerging from the pupal skin or while they are ovipositing. It should be noted that the more typical breeding places of *Simulium ochraceum,* the species believed to play the major role in the transmission of onchocerciasis in Guatemala, are too shallow and small to support development of *Profundulus punctatus,* the predatory minnow. Undoubtedly this minnow must reduce the blackfly population in the streams where it exists, but due to the enormous breeding potential of the flies and the constant reduction of the fish by the Indians who use them as food, the actual effect on the overall fly population must be small. However, in restricted areas which can be kept under supervision, this fish may be useful as a possible agent for biological control.

GUATEMALAN SPECIES OF SIMULIIDAE

Bequaert (1934) stated that up to the time of his investigations, he was able to find published records of only 3 Guatemalan Simuliidae. He discovered 3 additional species, bringing the total to 6. They are: *Simulium metallicum, S. mexicanum, S. exiguum, S. ochraceum, S. callidum,* and *S. rubicundulum.* In the valuable publication of Vargas (1945) 3 more species are listed: *S. haematopotum, S. pulverulentum,* and *Cnephia roblesi.* De León (1944, 1948) described 10 new species from Guatemala. Three of these have been put into synonymy by Vargas, Martínez and Díaz (1946) and Vargas and Díaz (1948) and one by Dalmat (1951). Nevertheless, since 3 of these synonyms were still new records for the country, the actual number of species was brought to 18.

The writer found 18 additional species to exist in Guatemala during the period 1948-1949, 9 of which he has described as new (Dalmat, 1949, 1950,

1951). He has also found one new record during the present year and 2 additional new species (1952b), thus bringing the total for Guatemala to 39. These are:

S. (*D.*) *acatenangoensis* Dalmat
Cnephia aguirrei Dalmat
Gigantodax (*G.*) *aquamarensis* (De León)
S. (*B.*) *benjamini* Dalmat
S. (*E.*) *aureum* Fries
S. (*H.*) *burchi* Dalmat
S. (*L.*) *callidum* Dyar & Shannon
S. (*H.*) *capricornis* De León
S. (*H.*) *carolinae* De León
S. (*L.*) *colvini* Dalmat
S. (*H.*) *delatorrei* Dalmat
S. (*L.*) *downsi* Vargas, Martínez & Díaz
S. (*L.*) *dugesi* Vargas, Martínez & Díaz
S. (*D.*) *earlei* Vargas, Martínez & Díaz
S. (*H.*) *ethelae* Dalmat
S. (*N.*) *exiguum* Roubaud
S. (*L.*) *haematopotum* Malloch
S. (*S.*) *jacumbae* Dyar & Shannon
S. (*S.*) *jobbinsi* Vargas, Martínez & Díaz
S. (*S.*) *kompi* Dalmat
S. (*H.*) *larvispinosum* De León
S. (*D.*) *mathesoni* Vargas
S. (*S.*) *metallicum* Bellardi
S. (*D.*) *mexicanum* Bellardi
S. (*H.*) *microbranchium* Dalmat
S. (*H.*) *nigricornis* Dalmat
S. (*S.*) *ochraceum* Walker
Cnephia pacheco-lunai (De León)
S. (*S.*) *parrai* Vargas, Martínez & Díaz
S. (*D.*) *pulverulentum* Knab
Cnephia roblesi (De León)
S. (*D.*) *rubicundulum* Knab
S. (*L.*) *samboni* Jennings
S. (*D.*) *smarti* Vargas
S. (*S.*) *tricornis* De León
S. (*L.*) *trivittatum* Malloch
S. (*L.*) *veracruzanum* Vargas, Martínez & Díaz
Gigantodax (*G.*) *wrighti* Vargas, Martínez & Díaz
S. (*D.*) *yepocapense* Dalmat

ANTHROPOPHILIC SPECIES

Of the above 39 species, the following 8 have been found to bite man in nature:

S. metallicum
S. ochraceum
S. callidum
S. exiguum
S. veracruzanum
S. haematopotum
S. downsi
Cnephia pacheco-lunai

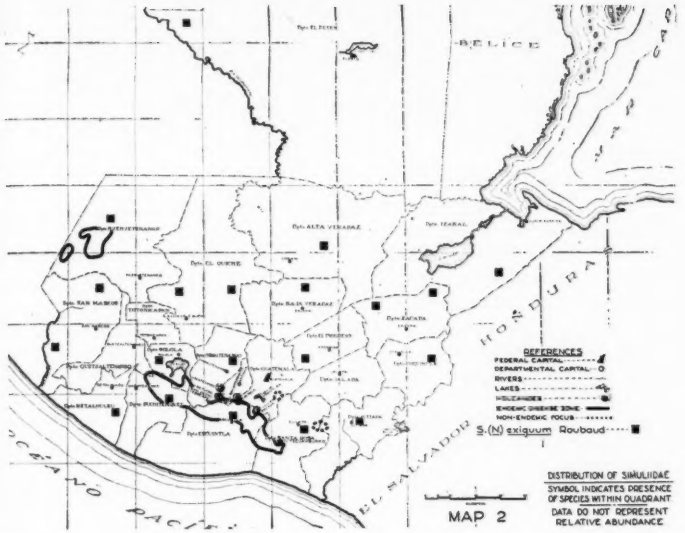
Of this group, *S. downsi* and *Cnephia pacheco-lunai* bite man only rarely, but *S. exiguum*, *S. veracruzanum*, and *S. haematopotum* are voracious biters of man. It has been found that at least *S. exiguum* and *S. veracruzanum* are widespread throughout large regions of the onchocerciasis zones (maps 2 and 3). Since *S. ochraceum*, *S. metallicum*, and *S. callidum* are usually found in the regions where the disease is prevalent, and since these species readily feed on man and are easily collected in the region of our field laboratory, most of our experimental work was done with them. This does not preclude the possibility that the other anthropophilic species may be involved in the transmission of onchocerciasis in at least some regions of the onchocerciasis zones. This will be further discussed under the section on natural and experimental infection.

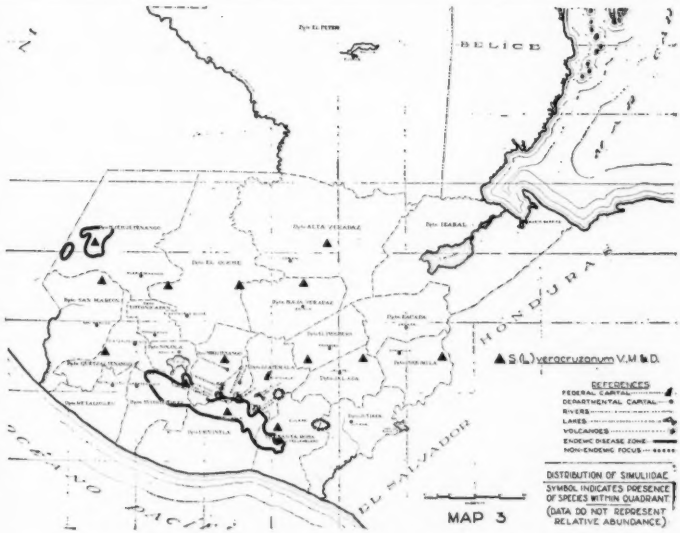
Table 1 summarizes the data on host preferences of the 3 principal anthropophilic species taken from experiments in which flies were collected from both human and other animal subjects that had been placed side by side. It will be noted that, considering the mammalian subjects in general, *S. ochraceum* demonstrated preponderantly anthropophilic habits, while *S. metallicum* and *S. callidum* showed preference for hosts other than human, or attacked both with almost equal avidity. It is primarily because of the decided preference of *S. ochraceum* for human hosts that it has been generally considered the vector, or principal vector, of onchocerciasis in the Western Hemisphere.

TABLE 1.—Biting preferences of principal anthropophilic species of *Simulium*. Comparison between man and other animal hosts. Each pair is comprised of two six-hour observation periods. "Total no. biting" represents the number of flies that were collected from both subjects during the two six-hour observation periods. The biting preference in each group is expressed as the percentage of the total number of flies biting man and the other animal subject.

Subject	Percent Biting *ochraceum*	*metallicum*	*callidum*
Man	85	10	8
Horse	15	90	92
Total no. biting	404	985	114
Man	99	26	31
Mule	1	74	69
Total no. biting	670	1,183	129
Man	100	15	44
Donkey	0	85	56
Total no. biting	205	772	34
Man	98	15	41
Cow	2	85	59
Total no. biting	812	1,033	27
Man	99	87	92
Pig	1	13	8
Total no. biting	870	255	25
Man	94	67	53
Sheep	6	33	47
Total no. biting	2,531	902	153
Man	90	54	69
Dog	10	46	31
Total no. biting	1,128	687	80
Man	78	61	40
Goat	22	39	60
Total no. biting	303	259	78
Man	99	98	100
Cat	1	2	0
Total no. biting	692	333	16
Man	99	94	100
Pigeon	1	6	0
Total no. biting	231	51	3
Man	100	80	100
Duck	0	20	0
Total no. biting	77	135	4
Man	99	96	94
Turkey	1	4	6
Total no. biting	1,077	424	33
Man	99	94	100
Chicken	1	6	0
Total no. biting	363	88	23

The epidemiological evidence, up to the time of our work, also supported this viewpoint since it indicated that *S. ochraceum* was found almost exclusively within the regions of the disease. However, although this species is found in almost all parts of the onchocerciasis zones, it is also very generally distributed outside of the zones (map 4). It will be noted on map 5 that *S. metallicum* has a distribution similar to that of *S. ochraceum*. Attention should be called to the fact that several other anthropophilic species are distributed throughout





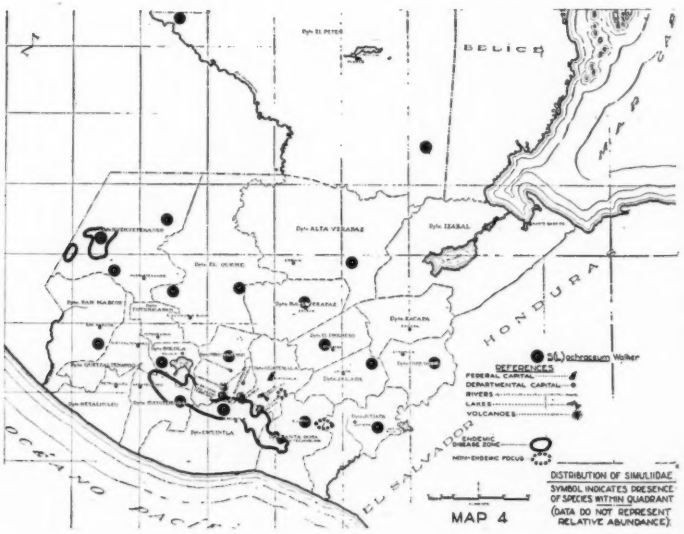
large regions of the onchocerciasis zones as well as outside of the zones. *S. callidum*, although found well distributed in the onchocerciasis zones (map 6), is always found in numbers so small that its importance in transmission has been discounted.

LIFE HISTORY AND ECOLOGY OF SIMULIUM VECTORS

The Simuliidae, or blackflies, are small nematocerous Diptera, the adults rarely exceeding 6 mm in length. They are often called "buffalo gnats" because of the "hump," or arch, that is characteristic of the thorax. The mouthparts are formed for piercing and sucking, but only the females are known to take blood.

The larval stage of all known species is passed in running water, varying from waterfalls to minute trickles, seldom in swampy areas or stagnant water. The larvae attach themselves to the substratum by means of an anal disc which makes it possible for them to retain their position even in very swift water. Larvae are usually found in the shallower parts of the streams, especially where obstructions cause rippling of the water surface. Rocks, stones, leaves, branches, and all sorts of debris serve as substrata for them. The larval stage lasts from 1 to 9 weeks, depending on the species and the environmental conditions. Five instars are usually reported. In the larval habitat, the last instar spins a silken cocoon in which pupation takes place. The shape of the cocoon is frequently used as a taxonomic character. The pupal stage varies from 2 to 10 days. Upon emergence, the adult rises to the surface of the water and takes to flight, soon after which mating is usually accomplished.

The longevity and flight ranges of the adults of some of the anthropophilic species in Guatemala have been reported as at least 85 days and 9.7 miles respectively by Dalmat (1952a). Oviposition usually takes place at the water's surface, or just below it, on some convenient substratum such as bare rock, emergent vegetation, or debris. At times the eggs are laid singly, but usually they are deposited in groups that are covered by a viscous matrix. A



MAP 4

single female may lay as many as 500 eggs. The young larvae hatch from the eggs in from 3 to 20 days.

In the following discussion of the biology and ecology of the vectors of onchocerciasis in Guatemala, only *S. ochraceum, S. metallicum,* and *S. callidum* will be considered.

SIMULIUM (S.) OCHRACEUM Walker

It has been particularly difficult to study the life history of this species because of its characteristic habitat and mode of oviposition. It is found breeding principally in *infant* and *young* streams which flow through "rugged" terrain. These streams are usually concealed by a dense canopy composed of 3 to 4 layers of vegetation: Emergent vegetation, overgrowth of grasses and other plants preferring a moist environment, shrubs and low trees, and tall trees. Thus, the breeding places are difficult to find and somewhat inaccessible. The species has been found at altitudes ranging from 500 to 6000 feet, with one collection at 8200 feet, but most commonly from 3000 to 5000 feet. At the breeding areas the streams show the following characteristics (table 2): *Width:* Range, 1 in-15 ft; optimum, 1-5 ft. Depth: Range, 1 in-3 ft; optimum, 1-5 in. *Temperature:* Range, 10-22°C; optimum, 18-20°C. *Current:* Range, 1-40 in per second; optimum, 1-10 in per second. *pH:* Range, <6-8; optimum, 7.1-7.5.

Eggs, larvae, and pupae have been found on parts of the following plants that were floating on the surface of the streams or were emergent from them: *Axonopus compressus* (Sw.) Beauv., *Tradescantia commelinoides* R. and S., *Tripogandra cumanensis* (Kunth) Woodson, *Ipomea* sp., *Hyptis sinuata* Pohl.

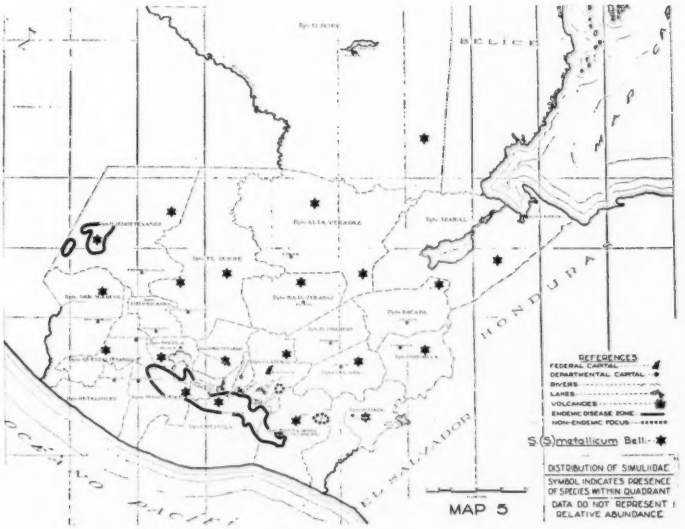
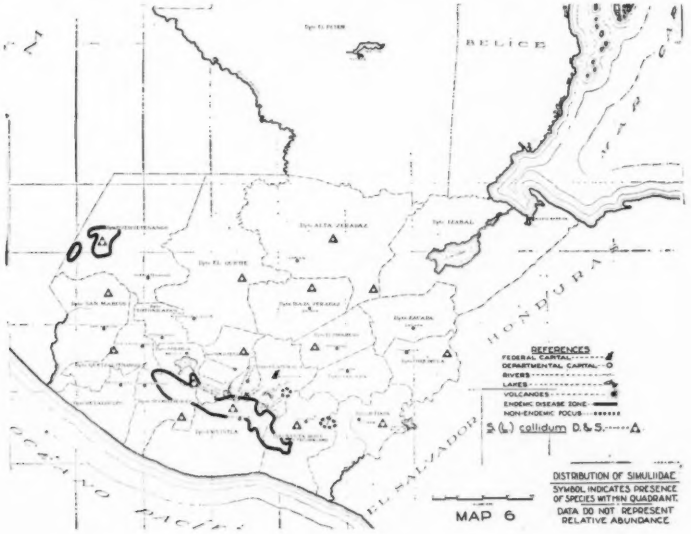


TABLE 2.—Ecological factors. Optimum Conditions for *S. ochraceum*, *S. metallicum*, and *S. callidum*

Species	Altitude (feet)	Stream Characteristics: Width (feet)	Depth (inches)	Temperature (°C)	pH	Current (inches/second)	Substratum
metallicum	3000-5000	1-5	1-5	18-20	7.1-7.5	1-10	Leaves & stems
ochraceum	2000-5000	1-8	<12	17-20	6.6-8.0	1-20	Leaves; stones
callidum	900-6000	1-15	1-12	17-20	6.6-8.0	1-30	Leaves; debris; stones

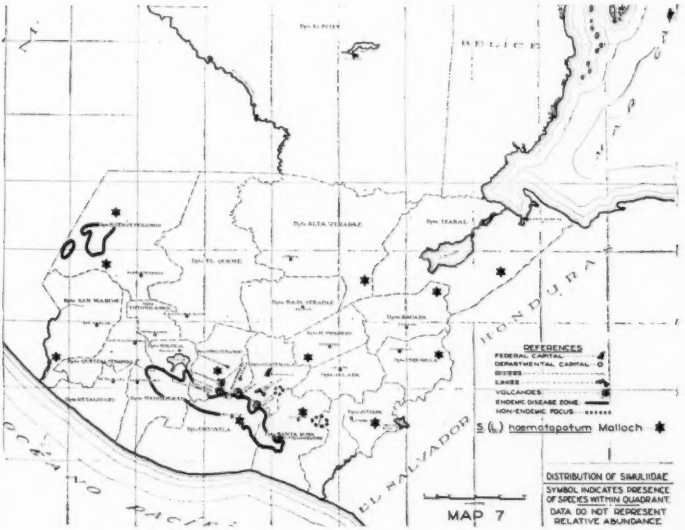
The immature stages have occasionally been found in debris vegetation, but never on hirsute plants. The following species of blackflies have been found breeding in the same streams with *S. ochraceum: S. metallicum, S. callidum, S. downsi, S. mexicanum, S. rubicundulum, S. acatenangoensis, S. veracruzanum, S. capricornis, S. smarti, S. parrai.*

The adults mate soon after emergence, but development of the eggs depends on the ability of the female to secure blood meals. The time required for eggs to develop within the female is not known. Fertilization takes place from the spermatheca as the eggs are laid. *S. ochraceum*, unlike the other two species considered here, oviposits by hovering above the less turbulent parts of the stream and dropping relatively few eggs in any one place on the floating emergent vegetation. It deposits 3 to 4 eggs in approximately 10 seconds. It is difficult to find eggs if the female is not found in the process of oviposition because so few are laid in any one place. It was not realized for a long time that the hovering female was ovipositing, since all other species observed in this process either entered the water to deposit



eggs, or approached or landed on a rock or a floating leaf. The oviposition has been observed most frequently between 12:00 n and 2:00 pm. The viscous matrix covering the eggs is of cream color, changing to brown and becoming harder within a few days. Within 3-10 days the young larvae emerge and soon migrate to an area where the current passes over the vegetation with somewhat more force. The larvae pass through 4 stadia, and probably a fifth, in 7-15 days. The more mature larvae maintain themselves in the swift currents but just prior to pupation, they migrate to more quiet sections of the stream, often on the underside of the leaves or stones where they are afforded more protection. Here the larvae spin the cocoon in which they pupate, the process taking about 5 hours. The adult emerges in from 4-6 days. From studies of longevity and flight range of flies captured in the field (Dalmat, 1952a), it was found that the females can live at least 27 days and can fly at least 6.3 miles, measured as a straight-line map distance. *S. ochraceum* is much more active in an atmosphere of higher humidity, and prefers more shaded situations, than do *S. metallicum* or *S. callidum*. It has often been collected while biting in the laboratory or in other buildings, both during the day and night. In the field, during the day, it has been found resting on the upper and lower surfaces of the leaves of several low trees and shrubs, especially *Polymnia maculata* Cav. and *Ricinus communis* L. Biting specimens have been collected in trees as high as 112 feet above the surface of the ground, and resting specimens as high as 51 feet. However, it prefers to remain within 50 or 60 feet of the ground level. At night, the adults have been found resting on the lower parts of the grasses and other similar vegetation, often close to the roots. The more salient characteristics of the life history and habits of *S. ochraceum* are summarized in table 3.

S. ochraceum is the most anthropophilic species in the principal oncho-



cerciasis zones of Guatemala. This is very clearly demonstrated in table 1 which compares its biting preferences when presented with the choice of either human or other animal subjects placed side by side. Although the species seems to prefer the upper region of the body (table 4), it will attack the lower regions as well if the upper parts are protected. The species is well distributed in most parts of the onchocerciasis zones (map 4), but it can also be found generally distributed in almost all parts of the country at altitudes within its usual range. It was not found in a small part of the onchocerciasis zone on the western frontier bordering Mexico (Department of Huehuetenango), where *S. veracruzanum* seemed to be the dominant human biter.

SIMULIUM (S.) METALLICUM Bellardi

This species is more adaptable to different types of breeding habitats than is either *S. ochraceum* or *S. callidum*. Larvae are found in *infant, young, adolescent,* and *mature* streams almost indiscriminately, and are often found breeding in temporary streams of only a few weeks' duration. The breeding sites range from 350 to over 9000 feet, and are either open to the sun or well shaded by trees and shrubs. Although the species is more commonly found breeding in regions from 2000 to 5000 feet, it is not uncommon in areas both below and above these limits. The following description of the habitat will help one understand the adaptability of the species (table 2): *Width:* Range, 1 in-15 ft; optimum, 1-8 ft. *Depth:* Range, 1 in->3 ft; optimum, <1 ft. *Temperature:* Range, 8-28°C; optimum, 17-20C°. *Current:* Range, 1 in per second—waterfalls; optimum, 1-2 in per second. *pH:* Range 6-8; optimum, 6.6-8.

TABLE 3.—Life history chart of the three principal anthropophilic species of simuliids of Guatemala

	Eggs			Larvae	Pupae
Species	No. per group Substratum	Hours of oviposition	Days to Hatching	Duration larval stages	Duration pupal stages
S. ochraceum	1-4 leaves	12:00 m-2:00 pm	3-10	7-15	4-6
S. metallicum	150-500 leaves	5:00 pm-6:30 pm	3-20	6-20	4-10
S. callidum	singly rocks	4:30 pm-5:30 pm	3-8	?	3-6

	Adults					
	Time of mating	Longevity (days)	Flight range (miles)	Height above ground flies biting	Host Preference	Regional preference on humans
S. ochraceum	soon after emerging	27	6.3	112 (0-60)*	man	upper torso
S. metallicum	soon after emerging	85	9.7	112 (0-112)*	horses, mules, cattle	lower torso
S. callidum	soon after emerging	20	3	112 (0-112)*	horses, mules, cattle	lower torso

* Generally preferred range.

The eggs, larave, and pupae have been found on the following plants: *Axonopus compressus* (Sw.) Beauv., *Coix Lachryma-Jobi* L., *Tradescantia guatemalensis* C. B. Clarke, *Tradescantia commelinoides* R. and S., *Tripogandra cumanensis* (Kunth) Woodson, *Heteranthera reniformis* R. and P., *Renealmia* sp., *Hyptis sinuata* Pohl, Leguminosae, Acanthaceae, Compositae.

Species of blackflies frequently found breeding along with *S. metallicum* are: *S. callidum, S. ochraceum, S. downsi, S. veracruzanum, S. yepocapense, S. rubicundulum, S. acatenangoensis, S. jobbinsi, S. jacumbae, S. capricornis, S. pulverulentum, S. smarti, S. parrai, S. carolinae.*

Like *S. ochraceum,* the adults mate very soon after emergence, the spermatozoa being stored in the spermatheca. Development of the eggs depends on blood meals. Fertilization occurs as the eggs pass the opening of the spermatheca in the process of oviposition. The female oviposits in two manners. If the current is very rapid, it approaches the appropriate leaf at the surface of the water and deposits an egg without apparently landing; it then hovers above the leaf and returns to the same spot to deposit the second egg. They are laid one in two seconds, contiguous to each other, but forming no general pattern and never overlapping. Should the stream be relatively slow-moving, the fly will actually land on the leaf to deposit the eggs. In this case, after each egg is laid, the fly moves about somewhat before returning to the area to lay the next egg. Often several females, at times as many as 30, have been seen ovipositing on a single leaf of *Renealmia* sp. One fly can deposit from 150 to 500 eggs in one mass. This species prefers the hours from 5:00 to 6:30 p.m. for oviposition although it has been observed on several occasions as early as 3:00 p.m. The first stage larvae emerge in from 3 to 20 days and soon arrange themselves in the smaller currents, most frequently attaching to leaves of plants, but at times, to stones and rocks. The larval development follows that of *ochraceum,* with pupation occurring after 6 to 20 days. The cocoon is woven on leaves or stones in a manner similar to that of *ochraceum.* If the case is on a flat surface, it bears a wing-like lateral extension on each side; if on rocks, inserted next to the vein on the underside of a leaf, or on a round twig, it may lack the extensions. The adult emerges in from 4 to 10 days. Longevity and flight range studies (Dalmat, 1952a) have shown that this species can survive at least 85 days in nature and can travel at least 9.7 miles, measured as a straight-line map distance. *S. metallicum* prefers lower humidity and bites more readily in the sun than does *S. ochraceum.* The day and night resting places are similar to those of *S. ochraceum.* Specimens have been encountered resting in trees and taking blood meals up to 112 feet from the ground level. The pertinent facts of the life history and habits of *S. metallicum* are summarized in table 3.

Although *S. metallicum* avidly attacks man, it prefers horses, mules, donkeys, and cattle (table 1). This species shows preference for the lower regions of the body when biting humans (table 4), but will also take blood readily from the upper regions when the lower parts of the body are covered.

S. metallicum is distributed through all parts of the onchocerciasis zones, but it has also been found throughout all other regions of the country. Its absence in various quadrants of the distribution map (map 5) is undoubtedly due to lack of sufficient collections in those areas. There are many parts of the onchocerciasis zones where this species is more prevalent than *S. ochraceum,* and other parts where *metallicum* exists and *ochraceum* is completely absent.

TABLE 4.—Simuliid feeding preference for body parts of human subjects. Upper and lower body parts of subjects exposed. Under general sunny conditions. Observations made on two different days from 6:30 am-5:00 pm. Preferences for particular body region expressed as percentage of total number of flies biting the subject.

Species	Total number biting flies	Per Cent Biting Upper Body Region					
		Face	Neck	Waist up front	Waist up back	Arm	Hand
S. ochraceum	785	2	3	24	40	8	2
S. metallicum	230	.5	.5	5	3	4	4
S. callidum	52	2	..	6	2	..	..

Species	Per Cent Biting Lower Body Region					Total Per Cent Biting	
	Upper leg	Shin	Calf	Ankle	Foot	Lower body region	Upper body region
S. ochraceum	9	3	8	1	....	79	21
S. metallicum	6	11	31	25	10	17	83
S. callidum	10	13	31	33	3	10	90

SIMULIUM (L.) CALLIDUM Dyar and Shannon

Although neither the pupae nor the adults of this species are found in large concentrations, it seems to have adapted itself to many different types of streams far better than *S. ochraceum*. It has been found breeding primarily in streams similar to those in which *S. metallicum* is found. Its unique oviposition habits and the migration of its larvae have made it difficult to study the life history of this species. The streams in which *S. callidum* are found are usually in regions not heavily wooded, and are only lightly shaded by trees and shrubs, if at all. They are invariably more accessible than the breeding places of *S. ochraceum*. The altitudes at which *S. callidum* has been taken are from 900 to 8200 feet, but usually between 900 to 6000 feet. The streams can be characterized as follows (table 2): *Width:* Range, 1 in->15 ft; optimum, 1-15 ft. *Depth:* Range, <1 in-3 ft; optimum, 1 in-1ft. *Temperature:* Range, 9-25°C; optimum, 17-20°C. *Current:* Range, 1 in per second—waterfalls; optimum, 1-30 in per second. *pH:* Range, <6->8; optimum, 6.6-8.

The eggs, larvae, and pupae have been found on the folloiwng plants: *Axonopus compressus* (Sw.) Beauv., *Coix Lachryma-Jobi* L., *Tradescantia commelinoides* R. and S., *Tradescantia guatemalensis* C. B. Clarke, *Tripogandra cumanensis* (Kunth) Woodson, *Renealmia* sp., *Ipomoea* sp., *Hyptis sinuata* Pohl, Leguminosae.

Other species commonly found breeding in the same streams as *callidum* are: *S. metallicum, S. ochraceum, S. downsi, S. exiguum, S. veracruzanum, S. rubicundulum, S. pulverulentum, S. yepocapense, S. smarti, S. capricornis.*

The mating, egg development, and fertilization of *S. callidum* are similar to those of the two other species just discussed. The females have been seen ovipositing only on sharply inclined surfaces of rocks and stones in parts of the stream where the current is not very strong, but where the water definitely flows over the eggs. The most usual hours were from 3:30 to 5:30 pm. The female hovers above the rock, lands only long enough to deposit one egg, then flies away to repeat the process on another suitable surface. On occasion,

females may deposits several eggs on one surface in an irregular group which is covered by a dark viscous material to which sand and minute particles of debris became attached. At times, 3 to 5 females have been observed in the process of oviposition in the same area. The individual eggs or egg groups can be found only after long and careful observation since they are well camouflaged by the dark viscous matrix. The young larvae emerge after 3 to 8 days and then migrate, approximately 50 per cent of the larvae attaching to leaves, the other 50 per cent choosing stones, twigs, and debris in about equal numbers. To date, it has not been possible to follow the larval development due to the oviposition habits and migrations of the larvae. The pupal stage lasts from 3 to 6 days, after which the adult emerges. Longevity and flight range studies (Dalmat, 1952a) have shown that the females of this species can survive at least 20 days in nature and that they can travel at least 3 miles without the aid of prevailing winds (table 4). The adults of this species prefer a relative humidity between 50 and 70 and a temperature between 17 and 19°C. The day and night resting places are the same as for *S. ochraceum* and *S. metallicum*. Forty-three feet above the ground flies were found resting on leaves of trees, and biting specimens were collected as high as 112 feet in trees. In table 3 the more important features of the life history and habits of *S. callidum* are summarized.

Although *S. callidum* commonly feeds on man, it definitely prefers horses, mules, donkeys, and cattle (table 1). When it does bite man, it shows definite preference for the lower body regions (table 4). The species is well distributed in most parts of the onchocerciasis zone and is also found in other parts of the country between 900 and 6000 feet of altitude (map 6).

NATURAL AND EXPERIMENTAL INFECTION

De León (1947) charted his results of dissections of wild-caught flies to show the percentage of natural infection with microfilariae of *Onchocerca volvulus*. In the month of December (coffee harvest time when laborers are most exposed to bites of *Simulium* spp.) he found 0.89 per cent of 433 *S. ochraceum* infected and 1.86 per cent of 631 *S. metallicum* infected on various fincas (plantations) of one of the onchocerciasis zones of Guatemala. Gibson (1951a) reported that, over a year period, he found natural infection in 0.38 per cent of 1,839 *S. ochraceum*, 1.04 per cent of 1,734 *S. metallicum*, and 0.62 per cent of 162 *S. callidum*. Gibson's data on natural infection rates, as well as data on experimental infection rates, are summarized in table 5. The data demonstrate that: *S. ochraceum, metallicum* and *callidum* will support the development of the human microfilariae and that most advanced development was observed in *S. metallicum*. Both *metallicum* and *callidum* appear capable of surviving in captivity for longer periods than *ochraceum*. Recently, Gibson (1951b) found an advanced larva in a specimen of *S. metallicum* which was dissected 310 hours after feeding on an infected person. This larva was in a pre-infective stage, thus adding additional evidence that *metallicum* can support the development of *O. volvulus*. Assuming that the species of *Onchocerca* is the same, the difference in the length of the developmental cycle of *O. volvulus* in the simuliid vector, as reported by Wanson, Henrard, and Peel (1946) in Africa and by Gibson (1951a, b) in Guatemala, may be due to the species of blackfly involved or to the effect of environmental factors.

The identity of the microfilariae in the flies was not given by De León or Gibson. As stated by the latter, skin biopsies of 884 local animals (mainly horses and cattle) showed that 12 per cent of the horses and cattle were infected with skin-inhabiting microfilariae, superficially similar to those of human onchocerciasis. There still remains the problem of developing adequate taxonomic characters with which to distinguish the species of microfilariae developing the flies. Since *S. ochraceum, S. metallicum,* and *S. callidum,* as well as attacking man, are also avid feeders on horses and cattle (table 1), it is quite possible that flies found infected in nature may have acquired their infection from the latter animals.

During a general survey of *Simulium* populations in all parts of Guatemala, some interesting observations were made in regard to the presence of certain of the anthropophilic species. It was noted that, in some areas of the nearly inaccessible onchocerciasis zone of Huehuetenango, *S. ochraceum* was completely absent and was apparently replaced by *S.* (*L.*) *veracruzanum* (map 3) as the principal anthropophilic species. In the lower altitude range of the Yepocapa zone, *S.* (*N.*) *exiguum* (map 2) became a dominant human biter while in other regions of the same zone *S.* (*L.*) *haematopotum* (map 7) seemed to replace *S. ochraceum* in importance. These findings recently stimulated a study of the potentiality of *S. veracruzanum, S. exiguum,* and *S. haematopotum* for transmitting onchocerciasis. Several hundred wild specimens of *S. exiguum,* caught in a region considered to be outside of the onchocerciasis zone, were dissected to determine whether this species carried any natural infection; a natural infection rate of 2.6 per cent was found in this group. Of 430 flies fed on a person known to have onchocerciasis and to be infective to flies, 20 per cent took up microfilariae and supported their development. One fly lived 13 days after taking its infective meal and contained filariid larvae in the preinfective stage. None of the examinations of 207 field workers, living in the region where the flies were captured, yielded positive biopsies. All of the biopsies taken from 12 horses were negative but 16 per cent of those taken from 75 cattle in the region were positive. It thus seems likely that *S. exiguum* naturally carries an *Onchocerca* sp. of cattle. Since, in the experimental studies, *S. exiguum* was found to support development of microfilariae of human onchocerciasis, this species can be considered a potential vector where it exists within the disease zone.

A similar study has been carried out with *S.* (*L.*) *veracruzanum* in an area where, although there are no autochthonous cases of onchocerciasis, there are numerous persons who have become infected while working during the coffee harvest on plantations in the disease zone. It is believed that the failure of the disease to become endemic in this region may be explained by the extreme dispersion of the people, who are primarily pastoral and live in isolated family groups. For this reason, the flies do not become infected with sufficient frequency to transmit the disease effectively. This is borne out by the failure to find natural infections in any of 415 wild specimens of *S. veracruzanum* that were examined. However, this species clearly demonstrated its potentiality as a vector when 29 per cent of 370 flies became infected after feeding on an infected individual. Some of these flies supported development of the microfilariae to at least the advanced sausage stage and showed much lower mortality due to infection than has been found in any other species of *Simulium.*

TABLE 5.—Comparison of natural and experimental infection rates with microfilariae of *Onchocerca volvulus*[1]

NATURAL INFECTION[2]

Days after feeding	*S. ochraceum* Number examined	*S. ochraceum* Infected with microfilariae	*S. ochraceum* Per cent infection	*S. metallicum* Number examined	*S. metallicum* Infected with microfilariae	*S. metallicum* Per cent infection	*S. callidum* Number examined	*S. callidum* Infected with microfilariae	*S. callidum* Per cent infection
0	1,839	7	0.38	1,734	18	10.9	162	1	0.62

EXPERIMENTAL INFECTION[3]

Days after feeding	*S. ochraceum* Number examined	*S. ochraceum* Infected with microfilariae	*S. ochraceum* Per cent infection	*S. metallicum* Number examined	*S. metallicum* Infected with microfilariae	*S. metallicum* Per cent infection	*S. callidum* Number examined	*S. callidum* Infected with microfilariae	*S. callidum* Per cent infection
1	285	126	44	198	86	43	22	15	68
2	387	181	47	342	162	47	38	16	42
3	115	44	38	247	84	34	29	17	59
4	39	19	49	241	110	46	15	2	13
5	2	0	0	93	13	14	8	0	0
6	4	0	0	35	1	3	5	0	0
7	....	....	....	21	1	5	4	0	0
8	....	....	....	32	5	16	....	....	....
9	....	....	....	8	1	13	1	0	0
10	....	....	....	2	0	0	....	....	....
11	....	....	....	....	....	....	1	0	0

[1] Adapted from Gibson.

[2] Wild flies collected in the field were immediately examined for presence of developing stages of microfilariae.

[3] Wild collected specimens of the three principal anthtrophilic species of *Simulium* were fed on an onchocercotic patient and held in the laboratory until they died, at which time they were examined for developmental stages of microfilariae.

S. haematopotum also demonstrated its vector potentiality when 7.5 per cent of 470 wild flies took up microfilariae after feeding on an infected individual, and two of these supported subsequent development of the larvae to the pre-infective stage (13 days). Natural infection was found in only 2 of 1276 wild specimens dissected. The species of *Onchocerca* was not determined. These data indicate that *S. exiguum, veracruzanum,* and *haematopotum* may have more significance in the transmission of onchocerciasis than is commonly conceded (Gibson and Dalmat 1952).

In view of these findings, it appears that the particular regions within a specific onchocerciasis zone may well have different vectors depending upon which of the anthropophilic species happens to be dominant in the area. This, in turn, depends largely on the ecological factors favoring breeding of a particular species.

Past experience has shown that insecticide control programs aimed at particular disease vectors, carried on with insufficient knowledge of the species involved, may not only result in failure, but also in formidable expenditure of money to control species not at all related to the transmission of the disease. With the data secured during the course of the study just reported, and after completion of laboratory tests to find suitable insecticides, it is expected that appropriate control measures against the vector species of onchocerciasis can be formulated.

SUMMARY

The present ecological study, part of a four-year investigation of the relation of the Simuliidae to the transmission of onchocerciasis in Guatemala, has been carried on in the Municipalidad of San Pedro Yepocapa, a highly endemic region of the principal disease zone.

The finding of *Simulium* spp. naturally infected with microfilariae of *Onchocerca volvulus,* the ability to infect flies with subsequent development of the microfilariae, plus the epidemiological evidence concerning the distribution of the anthropophilic species of *Simulium* in Guatemala, quite definitely incriminate them as vectors of the disease.

The larval stage of all known species of *Simuliidae* is passed in running water, varying from waterfalls to minute trickles, where they attach to stones, rocks, leaves, branches, or debris by means of an anal disc. The larvae pass through 5 instars in from 1 to 9 weeks, the last instar spinning the cocoon in which pupation takes place. The pupal period lasts from 2 to 10 days. The adults mate soon after emergence. The longevity and flight range of the adults of some of the anthropophilic species was found to be at least 85 days and 9.7 miles respectively. Oviposition usually takes place at the surface of the water, or just below it, on bare rock, emergent vegetation, or debris; the eggs, laid singly or in groups, are covered by a viscous matrix; the maximum number deposited by a single female is approximately 500. The young larvae hatch from the eggs in from 3 to 20 days.

The biology and ecology of *S. ochraceum, S. metallicum,* and *S. callidum,* the principal anthropophilic species in Guatemala, are treated in detail and summarized in tables 1, 2, 3, and 4 and on maps 4, 5, and 6.

Up to the time of this investigation, only 18 species of Simuliidae had been reported for Guatemala. The author since has found 21 additional species, 9 of which are described as new. Of the 39 species now known to exist in Guate-

maia, the following 8 show anthropophilic tendencies: *S. ochraceum, S. metallicum, S. callidum, S. exiguum, S. veracruzanum, S. haematopotum, S. downsi,* and *Cnephila pacheco-lunai.* Only the first 6 of these species commonly attack humans. Because of the decided preference of *S. ochraceum* for human hosts, it has generally been considered the vector, or principal vector, of onchocerciasis in the Western Hemisphere.

De León working on various fincas of an onchocerciasis zone in Guatemala during the month of December (season of greatest exposure to *Simulium* bites), found natural infection with microfilariae of *Onchocerca volvulus* on 0.89 per cent of 433 *S. ochraceum* and in 1.86 per cent of 631 *S. metallicum.* Gibson (1951a) reported over a one-year period, natural infection in 0.38 per cent of 1,839 *S. ochraceum,* 1.04 per cent of 1,734 *S. metallicum,* and 0.62 per cent of 162 *S. callidum.*

Gibson (1951a) traced the development of *O. volvulus* in *S. metallicum* up to 166 hours; in *S. callidum* up to 176 hours; and in *S. ochraceum* up to 48 hours after the flies had taken an infective blood meal. These studies of experimental infection, as well as of natural infection, are summarized in table 5.

It was found that in some areas of the onchocerciasis zones, *S. exiguum, S. veracruzanum,* and *S. haematopotum* replaced *S. ochraceum* as the principal anthropophilic species. Natural and experimental infection experiments carried on with these species showed that all three of them are capable of taking up microfilariae of *O. volvulus* and supporting their development.

Without accurate knowledge concerning the identity of the actual vectors of onchocerciasis, their breeding-places, life-history, and geographic distribution, attempted control by insecticides may fail. Much money and effort may be spent uselessly to control *Simulium* species which do not transmit the disease. The ecological data obtained during the study here reported provide a firm basis for efficient control.

After laboratory studies are made to find effective insecticides, it is expected that measures of control, appropriate against the species which are known to carry onchocerciasis, can be devised.

REFERENCES

BEQUAERT, J. C. 1929—The insect carrier of *Onchocerca volvulus* in Liberia. 4th Intern. Ent. Cong., Ithaca, 2, Trans. pp. 605-7.

———1934—*In:* Strong, R. P., J. C. Bequaert, J. D. Sandground and O. M. Muñoz. Onchocerciasis, with special reference to the Central American form of the disease. 234 pp. Harvard University Press.

BLACKLOCK, D. B. 1926—The further development of *Onchocerca volvulus* Leuckart in *Simulium damnosum* Theob. Ann. Trop. Med. Parasit. 20:203-18.

CALDERÓN, V. M. 1917—Enfermedad nueva en Guatemala; resumen de la conferencia dada por el Dr. Rodolfo Robles (Marzo 4, 1917). La Juventud Médica, Guatemala 17:97-116.

DALMAT, H. T. 1949—New species of Simuliidae (Diptera) from Guatemala-1. Ann. Ent. Soc. Amer. 42(4):538-553.

———1950—New species of Simuliidae (Diptera) from Guatemala-2. Ibid. 43(1):137-151.

———1951—Notes on the Simuliidae (Diptera) of Guatemala, including descriptions of three new species. Ibid. 44(1):31-58.

———1952a—Longevity and further flight range studies on the blackflies (Diptera, Simuliidae), using dye markers. Ibid. 45(1):23-37.

———1952b—Descriptions of two new species of *Simulium* (Diptera, Simuliidae) from Guatemala. Ibid. 45(2):339-347.

DE LEÓN, J. R. 1940a—Transmisión de la oncocercosis. Typed manuscript, 7 pp.

———1940b—Informe de los trabajos llevados a cabo por el Malariólogo de Sanidad Pública durante el año 1940. Typed manuscript.

———1944—Nevas especies de simúlidos en la región occidental de Guatemala. Bol. San. Guatemala 52:66-77.

———1947—Entomología de la oncocercosis. In: *Oncocercosis.* (Enfermedad de Robles). Univ. de San Carlos, Guatemala, 257 pp.

———1948—Un nuevo simúlido de la región occidental de Guatemala. Impreso No. 56 del Inst. Invest. Científicas, Univ. de San Carlos, Guatemala, 23 pp.

GIBSON, C. L. 1951a—Parasitological studies on onchocerciasis in Guatemala. Doctoral dissertation. University of Michigan, Ann Arbor, Mich. 97 pp.

———1951b—Personal communication.

——— AND H. T. DALMAT 1952—Three new potential intermediate hosts of human onchocerciasis in Guatemala. Am. J. Trop. Med. Hyg. 1(5):848-851.

HOFFMANN, C. C. 1930a—Nuevas investigaciones acerca de la transmisión de la oncocercosis de Chiapas. Rev. Mex. Biol. 10:131-140.

———1930b—La oncocercosis en el sur del Estado de Chiapas y su transmisión por el *Eusimulium mooseri.* Presented to the 7th Congreso Latinoamericano, México. 64 pp.

———1930c—Nota relativa a la transmisión de la oncocercosis. Hosp. Gen. Rev. Mex. 4:388-9.

———1930d—Investigaciones sobre la transmisión de la oncocercosis de Chiapas. An. Inst. Biol., México 1:59-62.

———1930e—Über *Onchocerca* im Süden von Mexiko und die Weiterentwicklung ihrer Mikrofilarien in *Eusimulicum mooseri.* Arch. f. Schiffs- u. Tropen-Hyg. 34(9):461-472.

———1931—Estudios entomológicos y parasitológicos acerca de la oncocercosis en Chiapas. Salubridad, México 3:669-97.

LEUCKART, R. 1893—Die Parasiten des Menschen und die von ihnen herrührenden Krankheiten. Ein hand- und Lehrbuch für Naturforscher und Aerzte. 2. Aufl., v. 1, 5. Lief, 2 Abt., viii+441-736 pp. Leipzig.

ROBLES, R. 1919—Onchocercose humaine au Guatémala produisant la cécité et l'érysipéle du litoral. Bull. Soc. Path. Exot. 12:442-60.

SHARP, N. A. D. 1927—A note on *Agamofilaria streptocerca* Macfie and Corson, 1922. Ann. Trop. Med. Parasit. 21:415-7.

STRONG, R. P. 1931a—*Onchocerca* investigations in Guatemala. Report of progress of the Harvard expedition. N. England J. Med. 204:916-920.

———1931b—Onchocerciasis in Guatemala. Preliminary Report. 20th Rept. United Fruit Co., Med. Dept. pp. 152-160.

———1931c—Onchocerciasis in Guatemala. Science (1900) 73:593-594.

———1934—Onchocerciasis, with special reference to the Central American form of the disease. Contrib. No. 6, Dept. Trop. Med. and Inst. Trop. Biol. and Med., Harvard Univ., Cambridge. 234 pp.

VARGAS, L. 1945—Simúlidos del Nuevo Mundo. Monografía núm. 1 del Inst. Salub. Enferm. Trop. México.

———1948—Notas sobre la oncocerciasis. VII. Infección experimental de *Simulium* (*Lanea*) *mangabeirai* con *Onchocerca volvulus.* Rev. Inst. Salub. Enf. Trop. 9:309-11.

———, AND A. DÍAZ NÁJERA 1948—Nuevas especies de México y consideraciones diversas sobre especies ya descritas. Ibid. 9(4):321-369.

———, A. MARTÍNEZ PALACIOS AND A. DÍAZ NÁJERA 1946—Simúlidos de México. Ibid. 7(3):101-192.

———, AND F. RUIZ REYES 1949—*Simulium exiguum* infectado con microfilarias de *Onchocerca volvulus.* An. Inst. Biol., Mexico 20:271-4.

WANSON, M., C. HENRARD AND E. PEEL 1946—*Onchocerca volvulus* Leuckart. Indices d'infection des simulies agressives pour l'homme. Cycle de développement chez *Simulium damnosum* Theobald. Recueil Travaux Sci. Med. au Conge Belge 4:122-138.

A New Species of Box-Mite from Colorado (Oribatei : Phthiracaridae)

Tyler A. Woolley
Colorado A & M College, Fort Collins

In one of his numerous papers on North American phthiracarid mites Jacot (1930, 1933, 1938) included a key to the species of the genus *Phthiracarus*. When this key was used to classify specimens of these mites from an area south of Estes Park, Colorado, it was found that they did not fit any of the descriptions. Careful comparison of the descriptions of the known species indicated that these specimens represented an undescribed species, for which the writer proposes the name *Phthiracarus minimarginatus*.

The new species differs from *P. bryobius* in its long, bristle-like pseudostigmatic organ, a ventral plate with an entire margin, not denticulate, and a projecting rostral rim. The rostral rim is distinct, although rather small, which suggests the name *minimarginatus*. In addition to the projecting aspal rim, the new species differs from *P. boresetosus* in the external insertions of both VP 1 and VP3. Bristle VP 2 is the only bristle of the ventral plate inserted in a deep notch. Other differences include six bristles on each genital cover, where *P. boresetosus* exhibits seven. The arrangement of the bristles on both the genital and anal covers differs markedly also.

The new species is accommodated in Jacot's key (1938) by extending the key one couplet, as follows:

1. Notogastral bristles longer than anal cover 2
1. Notogastral bristles shorter than anal cover 5
2. Five bristles on mesal rows (not including VP 3) 3
2. Six bristles on mesal rows ..... *P. setosus*
3. Aspis with projecting rim 4
3. Aspis without projecting rim ..... *P. boresetosus*
4. Pseudostigmatic organs short ..... *P. bryobius*
4. Pseudostigmatic organs long ..... *P. minimarginatus* sp. nov.
5. Pseudostigmatic organs long; notogastral bristles stout, not gradually tapering to a fine point, six in mesal rows ..... *P. olivaceous*
5. Pseudostigmatic organs short 6
6. Rostrum projecting beyond rim of aspis 7
6. Rostrum not projecting beyond aspal rim 8
7. Bristles medium long ..... *P. compressus*
7. Bristles very short and fine ..... *P. brevisetae*
8. Rim formed of reflexed edge of aspis; pseudostigmatic organs blunt ..... *P. anonymus*
8. Rim formed of thickened edge; pseudostigmatic organs pointed 9
9. Anterior end of aspis high, angular; notogastral bristles a:1 distant from collar ..... *P. sphaerulus*
9. Anterior end of aspis low, rounded; notogastral bristles a:1 on edge of collar ..... *P. setosellus*

In the following descriptions the writer used the system of terminology and bristle designation as found in Jacot (1938). A camera lucida was used for the execution of figs. 1 and 3. Fig. 2 is a free-hand sketch.

Phthiracarus minimarginatus sp. nov.

Figs. 1-3

Diagnostic characters.—Rostrum with slight, but distinct projecting rim; aspis broadly curved; length of pseudostigamtic organ as long as distance from dorsal edge of collar to ventral edge of notch, when viewed from side angled outward from insertion, curved slightly upward, projected forward to near level of insertion of rostral bristle, hispid at distal tip (fig. 2), resembling a long bristle. Insertions of VP 1 and VP 3 external, VP 2 inserted in a deep notch. As seen from below each genital cover with three marginal bristles (I:1, 2, 3) in anterior third of cover, aligned almost directly behind each other; bristles II:1, 2, 3 slightly laterad of bristles of row I, insertions in a straight line; II:1 inserted in a transverse plane which bisects the distance between I:2 and I:3. Bristles I:1 and I:2 of anal covers near margin and subequally spaced near center of cover length; II:1, 2, 3 laterad of bristles I:1 and I:2, but subequally spaced along longitudinal mid-line of each cover.

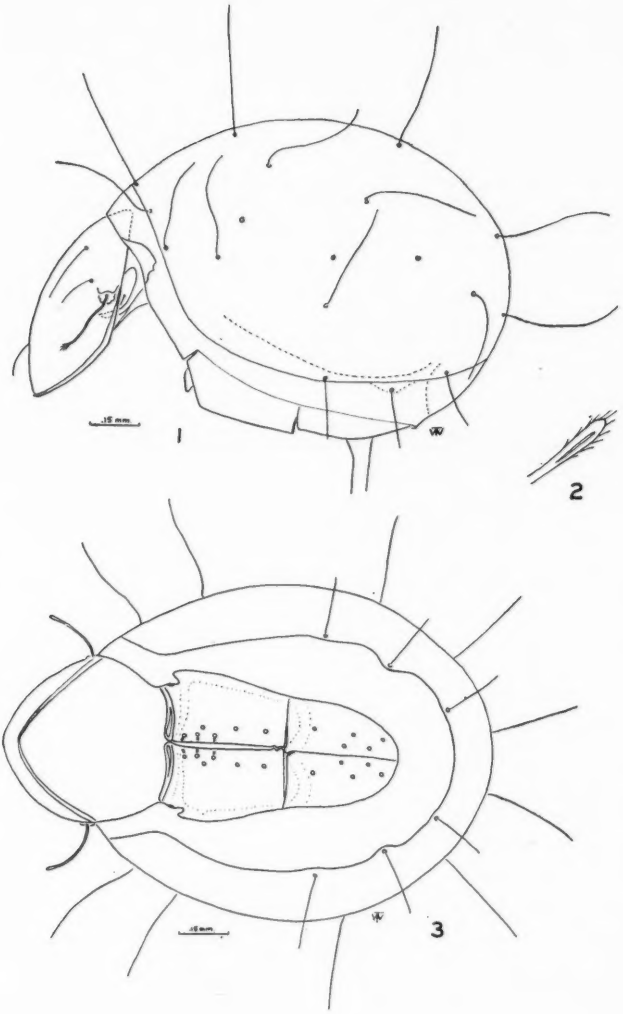
Dimensions.—The following measurements were obtained from ten specimens. These data represent minimum, intermediate and maximum measurements for notogaster dimensions. Length—428, 471, 485. Width—328, 357, 371. Height—314, 328, 342.

Description.—Surface of aspis finely vermiculate, profile of aspis as seen from side broadly rounded, dorsal face somewhat flattened, anterior and posterior ends decurved. Rim distinct, small near rostrum, projecting slightly beyond anterior edge of rostrum. Rostral bristles inserted nearly twice their lengths from anterior edge of aspis, decurved. Carina absent. Rib distinct, short, broad at base near rim, curved dorsad about its length from pseudostigmata. Lid distinct, covering approximately half of pseudostigmata as viewed from side, a shallow, curved notch at its center. Pseudostigmata rosette-like, depressed beneath lid. Pseudostigmatic organ as long as distance from dorsal edge of collar to ventral edge of notch, resembling a long, heavy bristle; as seen from the side, angled outward from insertion, curved dorsad slightly and projected forward to near level of insertion of rostral bristles, distal tip hispid. As seen from above pseudostigmatic organ projected almost directly laterad from insertion, the distal two-thirds angled forward slightly, distal third projecting mesad, thicker at tip. Insertion of lateral bristle the length of lid dorsad of pseudostigmatic organ, lateral bristle projecting forward, only slightly decurved. Vertical bristle nearly straight, inserted three-quarters of its length posteriad and mediad of lateral bristle. Nuchal bristle fine, inserted nearly its length directly below ventral edge of lid.

Surface of notogaster finely vermiculate, profile broadly oval as viewed from side, antero-dorsal margin decurved less abruptly than posterior margin, which curves downward and forward to meet the ventral plate; roundly oval as seen from above. Collar broad at dorsal limits, narrowing toward notch; notch elongate, denticulate at its ventral margin, denticles rounded; lapet broad, rounded near notch, angular at its lower end. Notogastral bristles as long as distance from dorsal edge of notch to ventral margin of lapet. Bristles of mesal rows erect, other bristles curved and bent (perhaps as a result of coverslip pressure). Bristle a:1 inserted near posterior margin of collar, a:2 and a:3 aligned parallel to posterior margin of collar, a:2 above level of dorsal edge of notch, a:3 inserted on level with center of notch; b:1 about its length posteriad of a:1, b:2 slightly greater than its length posteriad of a:2, somewhat higher in level of insertion than a:2, b:3 inserted below and between insertions of a:3 and b:2, closer to a:3; a pseudoforamina aligned almost directly between insertions of b:2 and b:3, closer to b:3; c:1 slightly more than its length posterior to b:1, c:2 less than its length ventrad and anteriad of insertion of c:1, c:3 its length ventrad and anteriad of c:2; a pseudoforamina nearly midway between insertions of c:2 and c:3; d:1 more than its length posteriad of c:1, closer to c:1 than c:1 is to b:1, d:2 less than its length ventrad and anteriad of d:1, almost on a level with e:1; a pseudoforamina midway between c:2 and d:2; e:1 inserted slightly ventrad of most posterior margin of notogaster, nearly on level with c:3.

Viewed from below ventral plate broadly rounded behind, margin entire, without denticles. Insertion of VP 1 slightly notched but insertion external, insertion of VP 2 within a deep notch, insertion of VP 3 external, with a very slight notch. Greater width of ventral plate at posterior junction with anal covers as viewed from side, narrowed anteriorly.

Genital covers convex, longer than broad, notched at antero-lateral margins to receive projections of ventral plate; accessory plate with a ventral horn or spine (fig. 1); nubbins slightly developed. Outline of plates curved on lateral margins as viewed from below, narrowed posteriorly. Three bristles in each row; bristles I:1, I:2 and I:3 in anterior



Figs. 1-3.—1. Lateral view of aspis and notogaster. 2. Free-hand sketch of distal tip of pseudostigmatic organ. 3. Ventral view of notogaster showing genital and anal covers and ventral plate.

third of cover, aligned almost directly behind each other; bristles II:1, II:2 and II:3 slightly laterad of bristles of row I and in a straight row; II:1 inserted on a transverse plane which bisects the distance between I:2 and I:3.

Anal covers convex, partially retracted into notogaster (fig. 1), lateral margins slightly indented posteriad of antero-lateral corners, broadly curved posteriorly; nubbins indistinct; bristles I:1 and I:2 near margin and subequally spaced near center of each cover; II:1, II:2 and II:3 laterad of marginal bristles and subequally spaced along midline of each cover, distance between II:1 and II:2 slightly greater than distance from II:2 and II:3.

Collection data.—Ten specimens were collected by the author from moss and grass under aspen at Mount Meeker Camp Ground, SE¼ S11, T3N, R73W, 6th principal base and 6th principal meridian, Boulder County, Colorado, July 17, 1952. The *holotype* (USNM 2110) and two *paratypes* (USNM 2086) were deposited at the U. S. National Museum. One additional *paratype* (CNM 6034) was deposited at the Canadian National Museum, Ottawa, Canada.

REFERENCES

JACOT, A. P. 1930—Oribatid mites of the subfamily Phthiracarinae of the northeastern United States. Proc. Boston Soc. Nat. Hist. 39(6):209-261.

———1933—Phthiracarid mites of Florida. Jour. Elisha Mitchell Sci. Soc. 48(2):232-267.

———1938—More box-mites of the northeastern United States. Jour. N. Y. Ent. Soc. 46(2):109-145.

Status and Synonymy of the American Percid Fish Hadropterus scierus*

Carl L. Hubbs
Scripps Institution of Oceanography, University of California, La Jolla
John D. Black
Northeast Missouri State Teachers College, Kirksville

There has been much disagreement in regard to the characters, classification, and nomenclature of the American darters described as *Hadropterus scierus* Swain (1883:252), as *H. scierus serrula* Jordan and Gilbert (1886:16), and as *H. maxinkuckiensis* Evermann (1900a:366). The questions involved have therefore been subjected to inquiry.

When we prepared the first draft of this paper more than ten years ago, we planned to present a detailed map and discussion of the distribution of *H. scierus;* to deal with its geographical and speciational relationships with other species, particularly *H. nigrofasciatus;* to treat in detail the geographical variations within *H. scierus;* and to describe a new subspecies from San Marcos River and other parts of the Guadalupe River system in Texas. The last two items have now been handled for the Southwest by Clark Hubbs (1954). The other tasks remaining undone we must leave to others.

ACKNOWLEDGMENTS

For use of material and other cooperation we are indebted to the authorities of the United States National Museum, the Museum of Zoology of the University of Michigan, and the Chicago Natural History Museum. Individuals who have lent us a helping hand include Reeve M. Bailey, J. L. Baughman, Aden C. Bauman, Clark Hubbs, Laura C. Hubbs, Leonard P. Schultz, and Boyd W. Walker.

SERRATED PREOPERCLE AND CLASSIFICATION OF HADROPTERUS SCIERUS

The character of the serrated preopercle, which has been stressed in the separation and classification of *Hadropterus scierus,* is much less distinctive than has often been indicated. This feature was not mentioned by Swain (1883) in the original description of the species, but was pointed out in the next year by Gilbert (1884:205), who gave the "well-developed serration of the preopercle" as the chief character of the new genus *Serraria,* based on Swain's species. Eigenmann and Fordice (1886:411) and Jordan and Gilbert (1886:16) stated that the serrations become obsolete in large adults. In reporting on additional material from Indiana Jordan (1890:165) wrote "preopercle serrulate, at least in young specimens." We find, however, that the serrulations are commonly lacking in the young and tend to become stronger with age (as noted by Meek, 1896:348). Jordan and Evermann (1896a:1037) qualified the character with these statements: "preopercle more or less distinctly serrate, especially in the young and in southern specimens." We find, however, that the range of variation is about the same in the south as in the north, except that the extreme conditions characterize the populations of different streams in Texas. Forbes and Richardson (1909:290) found no serrations in the two half-grown specimens they had from Illinois, and Evermann and Clark (1920:432) detailed the variation in this character, as exhib-

* Contributions from the Scripps Institution of Oceanography, New Series, No. 687.

ited by Indiana specimens. Statistical data on the number of serrae in the Southwest and in Indiana are being presented by Clark Hubbs (1954).

The rudimentary serration of the preopercle in a number of darters harks back to an ancestral condition, either directly or as the recrudescence of a character that had been phenotypically suppressed, like the ctenii on the scales of the poeciliid genus *Xenodexia* (Hubbs, 1950:5-7). (In describing the ctenoid scales of *Xenodexia* as unique among cyprinodonts, I forgot that the African lacustrine cyprinodontid genus *Lamprichthys* is similarly though presumably independently endowed. The same evolutionary reasoning probably holds for the origin of the ctenii in both genera, for in this respect each is unique in its family. There is a strong alternative possibility—that *Lamprichthys* is a primitive atherine that has lost its spinous dorsal.—C.L.H.)

The serrulations of the preopercle certainly provides no ground for the generic or even subgeneric separation of *Hadropterus scierus*. In this character *H. scierus* scarcely differs from the genotype of *Hadropterus* (*nigrofasciatus* Agassiz). As Boyd W. Walker pointed out to us, in some series of *nigrofasciatus* most of the half-grown to adult specimens have weak to strong preopercular denticulations. Some of the cotypes of that species have the preopercle serrulate and the type of *Alvordius spillmani*, a synonym, has definite though weak serrations.

H. scierus further agrees with *H. nigrofasciatus* in that the scales of the midventral line, except those between the pelvic fins, are hardly modified in the female and in the adult male are smaller and less spinose than in some other species. This character was not accurately stated and was given undue prominence in Jordan and Evermann's key (1896a:1029-1030).

The close relationship between *H. sciurus* and *H. nigrofasciatus* was appreciated by Swain (1883:252).

The moderate union of the gill-membranes, which led Swain (1883) to question the pertinence of *H. scierus* to the genus *Hadropterus* and which was used by Gilbert (1884:205) as another generic character of *Serraria* is a reasonably good specific character but varies too much intraspecifically as well as interspecifically to be used as a generic or subgeneric criterion. This variation has been shown by relative measurements, such as those presented by Hubbs and Raney (1939:4, Table I).

Typical *H. scierus*, from the Ohio River system in Indiana, Tennessee, and northern Alabama, differs from *H. nigrofasciatus* not only in the somewhat more connected gill-membranes and in coloration (more blotched, less banded), but also in having, on the average, more rays in the dorsal and anal fins (table 1). In Texas this distinction breaks down, for the new subspecies of the Guadalupe River system has an intermediate average number and other Texas races of *scierus* seem to agree with *nigrofasciatus* in this respect.

Both species occur, sometimes together, in the central part of the Gulf lowland, where their morphological and ecological relationships remain to be worked out. Since no indication of intergradation was observed, the species appear to be distinct.

STATUS OF THE NOMINAL TEXAS SUBSPECIES, SERRULA

The collections of this species from Texas have been referred to a distinct subspecies, *Hadropterus scierus serrula* Jordan and Gilbert (1886:16), with

TABLE 1.—Total number of dorsal plus anal rays in *Hadropterus nigrofasciatus* and in *H. scierus*

Species and State	Total number of dorsal plus anal rays									
	31	32	33	34	35	36	37	38	39	Aver.
H. nigrofasciatus										
Florida	....	....	5	8	3	1	....	....	....	34.0
Georgia	1	2	4	7	3	....	....	....	....	33.5
Alabama (cotypes)	....	....	1	1	1	....	....	....	....	34.0
Mississippi	....	1	2	8	5	2	1	....	....	34.4
H. scierus										
Indiana	....	....	....	....	2	4	3	4	....	36.7
Tennessee and N. Alabama	....	....	....	....	5	5	6	3	3	36.7
Texas, N. of Guadalupe R.	....	1	2	1	2	1	....	....	....	34.0
Texas, Guadalupe and San Marcos rivers	....	....	....	....	4	4	1	....	....	35.7

the following original characterization, repeated by Jordan and Evermann (1896a:1038) and other authors:

> Abundant. The Texas specimens of this species differ somewhat from those examined from Indiana and Arkansas, and may be taken as a distinct variety (*serrula*). The scales are somewhat smaller in variety *serrula* (lat. l. 68 to 71 in *serrula;* 64 to 66 in most Indiana examples). The coloration in *serrula* is paler, with more sharply-defined markings, the black blotches on the side being less confluent, and the sides of the belly without dark clouds.
>
> In the Texas specimens the breast is naked, while in most Indiana examples it is more or less scaly. The preopercle is very weakly, but generally distinctly serrulate.
>
> In very old specimens from Indiana these serrations disappear.

There is apparently some local variation in the number of scales, but neither in published counts (table 2) nor in original determinations (table 3) do we find grounds for the recognition of a fine-scaled Texas subspecies (except in that, on the average, the subspecies being named from the Guadalupe River system has more scales than other Texas races and slightly more than in typical northern *scierus*). The paler coloration attributed to the types of *serrula* was probably due to their having been taken in less clear water (specimens from the crystal waters of San Marcos River are very boldly marked). The degree of fusion of the lateral blotches is subject to much variation, as in other species of the genus, but with no apparent geographic trend.

TABLE 2.—Published scale counts for *Hadropterus scierus*

State	Scales	Reference	Identification (as given)
Indiana	65	Swain, 1883:252	*H. scierus*
Indiana	64-66	Jordan and Gilbert, 1886:16	*H. scierus*
Indiana	68	Jordan, 1890:160	*H. scierum*
Indiana	62-69	Evermann and Clark, 1920:434	*H. scierus*
Illinois	64-67	Forbes and Richardson, 1909:290	*H. scierus*
General	68-70 (scales), 64-71 (pores)	Jordan and Evermann, 1896a:1037	*H. scierus*
General	64-66	Jordan and Evermann, 1896a:1038	*H. scierus*
Arkansas	68-73	Meek, 1896:348	*H. scierum*
Texas	68-71	Jordan and Gilbert, 1886:16, and Jordan and Evermann, 1896a:1038	*H. scierus serrula*

TABLE 3.—Original scale counts for *Hadropterus scierus*

River and State	Number of scales in lateral-line series																		
	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	Aver.
Ohio R., Ohio and Kentucky	....	....	....	....	....	....	....	....	....	2	....	1	1	4	....	....	....	....	67.6
Indiana and Illinois*	1	1	1	4	6	11	11	10	8	14	8	7	5	2	3	2	....	....	63.8
Tennessee, Alabama, and Georgia	....	1	1	2	2	1	2	2	2	1	3	....	2	....	....	....	....	....	62.7
Mississippi R., Missouri	....	....	....	....	....	....	....	....	1	....	....	....	....	....	....	....	....	....	64?
St. Francis R., Arkansas	....	....	....	....	....	....	....	2	....	1	1	....	1	1	1	1	....	1	67.6
Arkansas R., Oklahoma	....	....	....	1	1	2	1	2	2	1	1	1	....	....	....	....	....	....	62.9
Red R., Arkansas	....	....	....	....	....	....	....	....	....	1	1	....	1	....	1	1	....	....	68.0
Sabine R., Texas	....	....	....	....	....	....	1	1	1	2	....	....	....	....	....	....	....	....	63.8
Neches R., Texas	....	....	....	....	....	....	....	....	1	....	....	....	....	....	....	....	....	....	64?
Trinity R., Texas	....	....	....	1	....	....	1	2	1	2	2	....	1	....	....	....	....	....	64.1
Buffalo Bayou and San Jacinto R., Texas	....	....	....	1	....	2	1	1	3	....	1	....	....	....	....	....	....	....	62.7
Lampasas R., Texas	....	....	....	....	7	4	5	11	7	2	4	1	....	....	....	....	....	....	62.8
San Marcos R., Texas	....	....	....	....	....	....	....	2	2	3	5	5	2	1	3	1	1	....	66.9
Guadalupe R., Texas**	....	....	....	....	....	....	....	....	....	1	1	3	....	4	1	1	....	....	68.1

* Only one from Illinois (Grand Pierre Creek), with 66 scales.
** From New Braunfels.

The blotching of the lower sides is often developed in adult females, at various localities. In all regions scales more or less cover the posterior half of the breast, especially toward the sides, and the anteromedian part of the breast is typically scaleless. The breast varies from wholly scaleless to com-

pletely scaled, but the extreme conditions seem rare everywhere. As previously mentioned, we find no marked geographical consistency in the degree of serration of the preopercle (except locally in Texas).

It is concluded that *Hadropterus scierus serrula* was not established on valid grounds (this view was indirectly indicated, in the statement of the range by Meek, 1894a:271 and by Pratt, 1935:119). Preliminary studies indicate tendencies toward local differentiation, but these appear too indefinite to be used now for the recognition of subspecies, other than the Guadalupe form. A more intensive study, based on an abundance of fresh material from throughout the wide range of the species, however, may lead to the conclusion that *Hadropterus scierus,* like many other darters, is a complex of local subspecies. The critical variational study being published by Clark Hubbs gives some basis for the separation of *serrula,* but since the work was largely confined to the Southwest, the author has refrained from recognizing any subspecies other than *H. s. scierus* and the Guadalupe form.

Since a subspecies *serrula* may eventually be recognized, it is desirable to fix the type locality. In the original account (1886) Jordan and Gilbert were equivocal in designating the type specimens. On page 16, in the section on "Sabine River at Longview, Texas" they made the following statement, under "*Hadropterus scierus* Swain, var. *serrula,* var. nov. (36481.)": "The Texas specimens of this species differ somewhat from those examined from Indiana and Arkansas, and may be taken as a distinct variety (*serrula*)." The characters thereupon assigned (see above) were taken from different lots. The smaller scales apply to the Guadalupe subspecies, whereas the "very weakly, but generally distinctly serrulate" preopercle best characterizes the material from Longview. Under "Trinity River, at Dallas, Texas" and "Rio Lampasas, at Belton, Texas," there are respectively listed (pp. 17-18), with ecological notes only, "*Hadropterus sicerus serrula* Jordan & Gilbert. (36476.)" and "*Hadropterus scierus* Swain (*serrula*)." The Rio Lampasas examples are labelled *H. scierus,* whereas those from the Trinity bear an unpublished manuscript name, but neither set is accompanied by an original label. The San Marcos specimens bear the same manuscript name, in Jordan's hand. Obviously, Jordan and Gilbert regarded all their Texas material as types of a form which they named *serrula* in the 1886 paper, but which, in fact, represents at least two subspecies. The restriction of the type locality seemingly was left to a subsequent author, apparently Evermann and Kendall (1894:88 and 113), who selected the Sabine River at Longview. Jordan and Evermann (1896a:1038 and 1896b:359) without warrant designated the type locality and type series as follows: "Jordan and Gilbert, Proc. U. S. Nat. Mus., 1886, 16, Red River, Fulton, Arkansas (Type, No. 36481. Coll. Jordan & Swain)." But Jordan and Gilbert did not collect the subspecies in Red River and No. 36481 came from the Sabine River. The heading for that river, set somewhat obscurely at the top of page 15, was obviously overlooked. (This slip was responsible for the inclusion by Jordan and Evermann of southern Arkansas in the range of *serrula.*) Hoping to remove doubt as to the validity of the type designation and type locality of *Hadropterus scierus serrula,* we hereby designate the series of specimens from the Sabine River at Longview, Texas (Cat. No. 36481, U. S. N. M.) as the sole lectocotypes. This action clears the way for the recognition of the Guadalupe subspecies and for the possible

eventual recognition of a subspecies from the Brazos River system, where the preopercular serrations appear to be more strongly developed than elsewhere. Other local characteristics of the Southwestern populations, particularly in proportions, are stressed by Clark Hubbs (1954).

SYNONYMY OF THE DUSKY DARTER

The following synonymy is designed to trace the complete nomenclatorial history of the species. It includes the references that pertain to the new subspecies being named from the Guadalupe River system in Texas (Clark Hubbs, 1954).

HADROPTERUS SCIERUS Swain

Hadropterus scierus.—Swain, 1883:252 (orig. desc.; close to *H. nigrofasciatus;* Bean Blossom Cr., Ind.). Jordan, 1885:867 (*Serraria* recognized as a subgenus). Jordan and Eigenman, 1885:69 (skeletal char.). Eigenmann and Fordice, 1886:411 (serrulations on preopercle obsolete in large adults). Jordan and Gilbert, 1886:13 (Washita [Ouachita] R. near Arkadelphia and Saline R. near Benton, Ark.; like Ind. specimens). Jordan and Evermann, 1896a:1020, 1037-1038 (desc.; range; syn.); 1896b:359 (range); 1900:3268, pl. 167, fig. 441 (Clinch R. at Walkers Ford, Tenn.). Evermann, 1900a:364-367 and 1900b:452 (Ind. records; *H. maxinkuckiensis* [erroneously] considered a local derivative). Osburn, 1901:92 (desc.; dist. in Ohio; Ohio R. near Little Sandy R.). Large, 1902:27 (char.; Skillet Fk., Wayne Co., Ill.). Eigenmann and Beeson, 1905:152 and Meek, 1908:170 (Ind. rec.). Forbes, 1909:389, 400 (dist., in Ill.). Forbes and Richardson, 1909 (and 1920):289-290, fig. 60 (desc.; Embarrass R., Ill.). Cockerell, 1913:154 (scale char.; Tippecanoe R., Marshland, Ind.). Hankinson, 1913:52 (Embarrass R. near Charleston, Ill.). Evermann and Hildebrand, 1916:449 (Clinch R., Tenn.). Evermann, 1918:321, 326, 355, 367 (rec. and dist., Ky. and Tenn.). Evermann and Clark, 1920:432-437 (Outlet Cr. below Lost L., Ind.; desc.; status *re H. aspro* and *H. maxinkuckiensis*). Pratt, 1923:124, 126 (diag.; range). Osburn, Wickliff, and Trautman, 1930:175 and Wickliff and Trautman, 1934:3 and 1937:3 (Ohio). Hubbs, 1946:38 (Okla.). Schrenkeisen, 1938:216-217 (desc.; range; relations). Trautman, 1940:29 (in key; Pike, Vinton, Jackson, and Gallia counties, Ohio; ecol.). Brind, 1941:65 (comp.). Lamb, 1941:45 (rec., San Jacinto R. system, Tex.). Baughmann, 1950:247 (Tex.). Moore and Rigney, 1952:11 (associated species; Blue R., Okla.). Moore, 1952:[10] (Okla.). Cross and Moore, 1952:407 (rec., Poteau R. syst., Okla. and Ark.). Hubbs, Kuehne, and Ball, 1953:237, fig. 20 (records mapped, upper Guadalupe R. syst., Tex.; subsp. undescribed).

Serraria sciera.—Gilbert, 1884:205 (desc.; type of new genus; Salt Cr., Ind.). Jordan, 1929:161 (desc.; range). Jordan, Evermann, and Clark, 1930:284 (range). ODonnell, 1935:489 (Embarrass R. near Charleston, Ill.). Pratt, 1935:119 (diag.; northern Ind. to Tenn. and Tex.). Blatchley, 1938:88 (char.; Ind. rec.; *H. maxinkuckiensis* a prob. var. [error]). Driver, 1942:284 and 1950:297 (char. in key; Ind. to Tex.).

Etheostoma scierum.—Evermann and Jenkins, 1888a:51, 54 and 1888b:119, 122, 123 (Ind. rec.). Jordan, 1888 (and 1890 to 1916):127-128 (desc.; range). Henshall, 1889:126 (Ohio R. near Little Sandy R.). Jordan, 1890:160, 164-165, 167 (char.; Ind. rec.). Gilbert, 1891:151-152 (Spring Cr., Courtland, Ala.). Woolman, 1892:260, 286, 288 (Ky. rec.). Hay, 1894:276 (desc.; Ind. rec.). Eigenmann and Beeson, 1894a:103-104 and 1894b:61 (Ind. rec.). Meek, 1894a:271 and 1894b:90-93 (Ark. ref.). Boulenger, 1895:80-81 (syn.; desc.; Bean Blossom Cr., Ind.). Meek, 1896:348 (Big Bay and Marked Tree, Ark.).

Hadropterus scieurus (misspelling).—Blatchley, 1901:257 (*H. maxinkuckiensis* prob. a var. [error]; Ind. rec.).

Hadropterus sciurus (misspelling).—Fowler, 1945:375 (specimens from San Marcos, Tex., differing from fig. of *H. "sciurus"* in physiognomy and color).

Hadropterus scierus scierus.—Shoup, Peyton, and Gentry, 1941:70, 73 (rec., Wolf and Obey rivers, Tenn.). Trautman, 1946:39 (in key; Pike, Vinton, Jackson, and Gallia counties, Ohio; ecol.). Gerking, 1946:85, 1 map (dist., rec., and ecol., Ind. [excluding rec. for L. Maxinkuckee]).

Hadropterus scierus serrula.—Jordan and Gilbert, 1886:16, 17, 19, 23, 24 (orig. diag.; Tex. rec.). Jordan and Evermann, 1896a:1038 (diag.; southern Ark. [error] through eastern Tex.); 1896b:359 (range repeated). Schrenkeisen, 1938:217 (color char.).

Etheostoma scierum serrula.—Jordan, 1888 (and 1890 to 1916):128 (char.). Evermann and Kendall, 1894:81, 84, 88, 93, 113 (rec. and dist. in Tex.). Evermann, 1918:321 (Bull Run, Hershells, Tenn.). Parks, Cory, and others, 1938:22 (Big Thicket area, east Tex.).

Hadropterus serrula.—Fowler, 1933:62 (Clear Cr., Reeves, La.).

Serraria sciera serrula.—Jordan, 1929:161 (char.).

Serraria serrula.—Jordan, Evermann, and Clark, 1930:284 (range).

STATUS OF HADROPTERUS MAXINKUCKIENSIS

A supposedly close relative and local representative of *Hadropterus scierus* was described from an inlet of Lake Maxinkuckee in Indiana by Evermann (1900a:366-367, pl. 17, lower fig.), under the name of *Hadropterus maxinkuckiensis*. This account was repeated, with modifications, by Evermann (1900b:452), Jordan and Evermann (1900:3166-3167), and Evermann and Clark (1920:435-437, 1 fig.). The nominal species was listed as distinct by Jordan, Evermann, and Clark (1930:284) under the name of *Alvordius maxinkuckiensis*. Holding to the name *Hadropterus maxinkuckiensis*, Blatchley (1901:257 and 1938:88, 1 fig.) regarded this nominal form as probably a variety of *H. scierus*, and Schrenkeisen (1938:217) has recently repeated Evermann's characterization. Fowler (1945:375) synonymized *H. maxinkuckiensis* with "*H. sciurus*." Gerking (1945:map 78) included a Lake Maxinkuckee record on his map for *H. s. scierus*.

An examination of the type specimen, description, and figure, however, suffices to show that *Hadropterus maxinkuckiensis* was based on a specimen of *Hadropterus maculatus* Girard (on the status of *H. maculatus* refer to Hubbs, 1926:59-60). In the color of the head, body, and fins, and particularly of the caudal base, as well as in the smooth preopercle and free gill-membranes, the type of *maxinkuckiensis* agrees with *H. maculatus* and differs from *H. scierus*. The scales are larger than usual in either species, but within the range of variation exhibited by each. The union of the dorsal fins and the large mouth in the type of *H. maxinkuckiensis* also fit into the limits of variation known for *H. maculatus*. That species shows much fluctuation in color pattern (but to date has defied analysis into subspecies). Consequently, *H. maxinkuckiensis* is to be relegated to the simple synonymy of *H. maculatus*.

REFERENCES

BAUGHMAN, J. L. 1950—Random notes on Texas fishes. Part II. Texas Jour. Sci. 2:242-263.

BLATCHLEY, W. S. 1901—The fishes of Lake Maxinkuckee. Ann. Rept. Dept. Geol. and Nat. Res. Ind. 25(1900):252-258.

———1938—The fishes of Indiana. Nature Publ. Co., Indianapolis:1-121, 38 figs.

BOULENGER, GEORGE ALBERT 1895—Catalogue of the perciform fishes in the British Museum. Cat. Fishes Brit. Mus. (2nd ed.) 1:i-xix, 1-294, pls. 1-15, figs. 1-27.

BRIND, WALTER LANNOY 1941—Encyclopedia of tropical fish. Mugilidae (the mullets). All-Pets Mag. 13:64-65.

COCKERELL, T. D. A. 1913—Observations on fish scales. Bull. U. S. Bur. Fish. 32(1912):117-174, pls. 32-40, figs. 1-52.

CROSS, FRANK BERNARD AND GEORGE A. MOORE 1952—The fishes of the Poteau River, Oklahoma and Arkansas. Amer. Midl. Nat. 47(2):396-412.

DRIVER, ERNEST C. 1942—Name that animal. A guide to the identification of the common land and fresh-water animals of the United States, with special reference

to the area east of the Rockies. Kraushar Press, Northampton, Mass:1-527, many figs.

———1950—The same (revised). *Ibid.*:1-558, many figs.

EIGENMANN, CARL H. AND CHARLES H. BEESON 1894a—The fishes of Indiana. Proc. Ind. Acad. Sci. 1893:76-108.

——— AND ———1894b—The fishes of Indiana. Rept. Ind. Comm. Fish. and Game 1894:40-64.

——— AND ———1905—The fishes of Indiana. *Ibid.* 1903-04:113-157.

——— AND MORTON W. FORDICE 1886—A catalogue of the fishes of Bean Blossom Creek, Monroe County, Indiana. Proc. Acad. Nat. Sci. Phila. 1885:410-411.

EVERMANN, BARTON WARREN 1900a—Descriptions of two new species of darters from Lake Maxinkuckee, Indiana. Rept. U. S. Comm. Fish and Fish. 1899:363-367, pl. 17.

———1900b—Some observations concerning species and subspecies. Science (n. s.) 40:451-455.

———1918—The fishes of Kentucky and Tennessee: A distributional catalogue of the known species. Bull. U. S. Bur. Fish. 35(1915-16):295-368.

——— AND HOWARD WALTON CLARK 1920—Lake Maxinkuckee. A physical and biological survey. Dept. Cons. St. Ind., Pub. 7, Vol. 1:1-660, many figs.

——— AND SAMUEL F. HILDEBRAND 1916—Notes on the fishes of east Tennessee. Bull. U. S. Bur. Fish. 34(1914):431-451, figs. 1-13.

——— AND OLIVER P. JENKINS 1888a—Notes on Indiana fishes. Proc. U. S. Nat. Mus. 11:43-57.

——— AND ———1888b—Notes on Indiana Fishes. Rept. Ind. Fish. Comm. 1888:108-127.

——— AND WILLIAM C. KENDALL 1894—The fishes of Texas and the Rio Grande basin, considered chiefly with reference to their geographic distribution. Bull. U. S. Fish. Comm. 12(1892):57-126, pls. 10-50.

FORBES, STEPHEN ALFRED 1909—On the general and interior distribution of Illinois fishes. Bull. Ill. St. Lab. Nat. Hist. 8:381-437, maps 1-103.

——— AND ROBERT EARL RICHARDSON 1909—The fishes of Illinois. Nat Hist. Surv. Ill. 3:i-cxxxi, 1-357, many figs. and pls.

——— AND ———1920—The same (2nd ed.). *Ibid.* 3:i-cxxxi, 1-357, many figs. and pls.

FOWLER, HENRY W. 1933—Notes on Louisiana fishes. Proc. Biol. Soc. Wash. 46:47-64.

———1945—A study of the fishes of the southern Piedmont and Coastal Plain. Monogr. Acad. Nat. Sci. Phila. 7:i-vii, 1-408, figs. 1-313.

GERKING, SHELBY D. 1945—The distribution of the fishes of Indiana. Investigations of Indiana Lakes and Streams 3(1):1-137, maps 1-113.

GILBERT, CHARLES H. 1884—A list of fishes collected in the East Fork of White River, Indiana, with descriptions of two new species. Proc. U. S. Nat. Mus. 7:199-205.

———1891—Report of explorations made in Alabama during 1889, with notes on the fishes of the Tennessee, Alabama, and Escambia rivers. Bull. U. S. Fish Comm. 9(1889):143-159, pl. 43.

HANKINSON, T. L. 1913—Distribution of fish in the streams about Charleston, Illinois. Trans. Ill. Acad. Sci. 6:102-113, 1 map.

HAY, O. P. 1894—The lampreys and fishes of Indiana. Ann. Rept. Ind. Dept. Geol. and Nat. Res. 19:146-296.

HENSHALL, JAMES A. 1889—Contributions to the ichthyology of Ohio. No. 2. Jour. Cincinnati Soc. Nat. Hist. 11:122-126.

HUBBS, CARL L. 1926—A check-list of the fishes of the Great Lakes and tributary waters, with nomenclatorial notes and analytical keys. Univ. Mich. Mus. Zool. Misc. Publ. 15:1-77, pls. 1-4.

———1946—List of the fishes of Oklahoma. In: A. D. Aldrich, Fish management guide for Oklahoma. Okla. Game and Fish Comm., Oklahoma City:36-39.

———1950—Studies of cyprinodont fishes. XX. A new subfamily from Guatemala, with ctenoid scales and a unilateral pectoral clasper. Misc. Publ. Mus. Zool. Univ. Mich. 78:1-28, map 1, pls. 1-4.

——— AND EDWARD G. RANEY 1939—*Hadropterus oxyrhynchus*, a new percid fish from Virginia and West Virginia. Occ. Pap. Mus. Zool. Univ. Mich. 396:1-9, pl. 1.

HUBBS, CLARK 1954—A new Texas subspecies, *apristis*, of the darter *Hadropterus scierus*, with a discussion of variation within the species. Amer. Midl. Nat.

———, ROBERT A. KUEHNE AND JACK C. BALL 1953—The fishes of the upper Guadalupe River, Texas. Tex. Jour. Sci. 5(2):216-244, figs. 1-34.

JORDAN, DAVID STARR 1885—A catalogue of the fishes known to inhabit the waters of North America, north of the Tropic of Cancer, with notes on the species discovered in 1883 and 1884. Rept. U. S. Fish Comm. 13:787-973.

———1888 (and subsequent printings and editions from 1890 to ed. 12, 1916)—A manual of the vertebrate animals of the northern United States including the district north and east of the Ozark Mountains, south of the Laurentian Hills, north of the southern boundary of Virginia, and east of the Missouri River inclusive of marine species. (5th ed.). A. C. McClurg and Co., Chicago:1-375.

———1890—Report of explorations made during the summer and autumn of 1888 in the Alleghany region of Virginia, North Carolina, and Tennessee, and in western Indiana, with an account of the fishes found in each of the river basins of those regions. Bull. U. S. Fish Comm. 8(1888):97-173, pls. 13-15.

———1929—Manual of the vertebrate animals of the northeastern United States inclusive of marine species. (13th ed.). World Book Co., Yonkers, N. Y.:i-xxxi, 1-446.

——— AND CARL H. EIGENMAN [EIGENMANN] 1885—Notes on skeletons of Etheostomatinae. Proc. U. S. Nat. Mus. 8:68-72.

——— AND BARTON WARREN EVERMANN 1896a—The fishes of North and Middle America. . . . Part I. Bull. U. S. Nat. Mus. 47(1):i-lx, 1-1240.

——— AND ———1896b—A check-list of the fishes and fish-like vertebrates of North and Middle America. U. S. Comm. Fish and Fish., Rept. Comm. 1895:207-584.

——— AND ———1900—The fishes of North and Middle America. . . . Part IV. Bull. U. S. Nat. Mus. 47(4):i-ci, 3137-3313, pls. 1-392.

———, ———, AND HOWARD WALTON CLARK 1930—Check list of the fishes and fish-like vertebrates of North and Middle America, north of the northern boundary of Venezuela and Colombia. Rept. U. S. Fish Comm. 1928 (pt. 2):1-670.

——— AND CHARLES HENRY GILBERT 1886—List of fishes collected in Arkansas, Indian Territory, and Texas, in September, 1884, with notes and descriptions. Proc. U. S. Nat. Mus. 9:1-25.

LAMB, LEONARD D. 1941—A preliminary fisheries survey of the San Jacinto River watershed. Trans. Tex. Acad. Sci. 24(1940):42-48, 1 map.

LARGE, THOMAS 1902—A list of the native fishes of Illinois with keys. Rept. Ill. St. Bd. Fish Comm. 1900-02 [App.]:1-30, 1 col. pl.

MEEK, SETH EUGENE 1894a—A catalogue of the fishes of Arkansas. Ann. Rept. Geol. Surv. Ark. 2 (1891):215-276, pls. 1-12.

———1894b—Report of investigations respecting the fishes of Arkansas, conducted during 1891, 1892, and 1893, with a synopsis of previous explorations in the same state. Bull. U. S. Fish Comm. 14:67-94.

———1896—A list of fishes and mollusks collected in Arkansas and Indian Territory in 1894. Ibid. 15(1895):341-349.

———1908—List of fishes known to occur in the waters of Indiana. Bien. Rept. Ind. Comm. Fish. and Game 1907-08:134-171.

MOORE, GEORGE A. 1952—A list of the fishes of Oklahoma. State Fish and Game Dept. Okla: 12 pp., 16 figs.

——— AND CARL C. RIGNEY 1952—Taxonomic status of the percid fish *Poecilichthys radiosus*, in Oklahoma and Arkansas, with the description of two new subspecies. Copeia 1952(1):7-15, pls. 1-2.

O'DONNELL, JOHN 1935—Annotated list of the fishes of Illinois. Ill. Nat. Hist. Surv. Bull. 20:473-500, figs. 1-4, 1 pl.

OSBURN, RAYMOND C. 1901—The fishes of Ohio. Ohio St. Univ., Univ. Bull. (5)20:105.

———, EDWARD L. WICKLIFF AND MILTON B. TRAUTMAN 1930—A revised list of the fishes of Ohio. Ohio Jour. Sci. 30:169-176.

PARKS, H. B., V. L. CORY AND OTHERS 1938—The fauna and flora of the Big Thicket

area (cover title: Biological survey of the east Texas Big Thicket area), 2nd ed., Huntsville, Texas:1-51, 9 figs.

PRATT, HENRY SHERRING 1923—A manual of land and fresh water animals of the United States (exclusive of birds). P. Blakiston's Son & Co., Philadelphia:i-xv, 1-422, figs. 1-184, 1 map.

———1935—The same, 2nd ed. *Ibid.*:i-xvii, 1-416, figs. 1-184, 1 map.

SCHRENKEISEN, RAY (edited by J. T. Nichols and F. R. LaMonte) 1938—Field book of fresh-water fishes of North America north of Mexico. G. P. Putnam's Sons, New York:1-312, 1 pl., 97 figs.

SHOUP, C. S., J. H. PEYTON AND GLENN GENTRY 1941—A limited biological survey of the Obey River and adjacent streams in Tennessee. Rept. Reelsfoot Lake Biol. Sta. 5 (= Jour. Tenn. Acad. Sci. 16(1) = Tenn. Dept. Conserv. Div. Fish and Game Misc. Publ. 3):48-76.

SWAIN, JOSEPH 1883—A description of a new species of Hadropterus (Hadropterus scierus) from southern Indiana. Proc. U. S. Nat. Mus. 6:252.

[TRAUTMAN, MILTON B.] 1940—Artificial keys for the identification of the fishes of the state of Ohio. Franz Theodore Stone Laboratory, Put-in-Bay, Ohio:1-38 (processed).

TRAUTMAN, MILTON B. 1946—The same. Ibid.:1-52 (processed; revised).

WICKLIFF, EDWARD L. AND MILTON B. TRAUTMAN 1934—List of the fishes of Ohio. Bull. Bur. Sci. Res., Div. Conserv., Dept. Agri. Ohio 1:1-4 (processed).

——— AND ———1937—The same [revised edition]. *Ibid.* 1:1-4 (processed).

WOOLMAN, ALBERT J. 1892—Report of an examination of the rivers of Kentucky, with lists of the fishes contained. Bull. U. S. Fish Comm. 10(1890):249-288, pl. 51.

A New Texas Subspecies, apristis, of the Darter Hadropterus scierus, with a Discussion of Variation Within the Species

Clark Hubbs
University of Texas, Austin

In this paper a new subspecies of darter from Texas is described, its ecology is discussed briefly, and a study is presented of the geographical variation exhibited by *Hadropterus scierus*, especially in Texas and Oklahoma.

Description of a New Subspecies of Darter from Texas

During the past two decades a number of Texas freshwater fishes have been recognized as meriting new taxonomic ranking. Recently four of these fishes have been named (Hubbs, 1951, and Hubbs and Bonham, 1951). In this paper a continuation of the series, a new darter is defined.

The methods of counting and measuring used in this investigation are those proposed by Hubbs and Lagler (1947). The variational data are expressed by a simplified graphical scheme that follows the Hubbs and Perlmutter (1942) modification of the method proposed by Dice and Leraas (1936). In each unit of the graphs the extreme range is represented by the base line, the mean by the vertical line, two standard errors on each side of the mean by the solid area, and one standard deviation on each side of the mean by the outlined area. This further modification of the Dice and Leraas method for the graphical comparison of series of samples was developed jointly with Carl L. Hubbs and is discussed in another paper (Hubbs and Hubbs, 1953).

The status and synonymy of the species *Hadropterus scierus* is being treated by Hubbs and Black (1954).

Acknowledgements

Drs. Carl L. Hubbs, John D. Black, and Reeve M. Bailey, all who had, either jointly or separately, prepared manuscripts to describe this form, kindly dropped their plans in favor of this paper. Drs. Bailey and Hubbs contributed helpful suggestions during the preparation of the manuscript. From the collections of the University of Michigan Museum of Zoology Dr. Bailey made available all Indiana specimens used in this study and many from scattered localities throughout Texas. Dr. George A. Moore provided the Oklahoma specimens studied. Mr. Kenneth C. Jurgens, Mr. Robert J. Kemp, Mr. R. Kirk Strawn, and Prof. Frank T. Knapp secured specimens from Texas. Mr. Marion Toole kindly gave permission for the author and his students to make numerous collections throughout Texas.

Hadropterus scierus apristis Carl L. Hubbs and Clark Hubbs n. subsp.—Guadalupe Dusky Darter

Hadropterus scierus serrula Jordan and Gilbert, 1886:23-24 (San Marcos and New Braunfels records). Jordan and Evermann, 1896:359 (same records). *Etheostoma scierum serrula* Evermann and Kendall, 1894:113-114 (San Marcos and New Braunfels records). *Serraria serrula* Jordan, Evermann, and Clark, 1930:284 (in part). *Hadropterus scierus* Baughman, 1950:247 (in part). Hubbs, Kuehne, and Ball, 1953:237. *Hadropterus sciurus* (misspelling) Fowler, 1945:375 (San Marcos specimens).

Diagnosis.—The preopercular serrae number 0 to 1, rarely 2 to 6. Fin rays: dorsal X to XIV (usually XI to XIII)-11 to 13 (rarely with one spine in front of soft dorsal

rays); anal II (rarely I), 7 to 10 (usually 8 or 9); caudal 15 to 17; pectorals 13 to 15 (rarely 12); pelvics I, 5. Scales: lateral-line 63 to 78 (usually 66 to 72); rows from dorsal to lateral line 8 to 10. Branchiostegals 6. Gill-rakers 1+9.

The mouth is inferior and oblique. The frenum is broad and without a groove. The upper jaw extends to a point below the most anterior part of the eye. The moderate eye, which approximately equals the snout and upper jaw lengths, barely reaches the dorsal profile. The jaws bear an outer row of enlarged teeth and an inner band of smaller teeth. Those forming a patch on the vomer and a row on each palatine are of the smaller size. The distance from the insertion of the pelvic fin to the union of the gill-membranes is contained 0.7 to 1.1 times in the distance from the union of the gill-membranes to the tip of the mandible (larger figures for smaller specimens). Eye length contained 1.8 to 2.2 times in distance from union of gill-membranes to tip of mandible (larger figures for larger specimens).

The profile rises gently from the rounded snout to the first dorsal spine, except for a slight hump at the nape. It remains more or less straight along the spinous dorsal and starts to decline to the level caudal peduncle either at the beginning or middle of the soft dorsal.

Except for the shorter last one or two, the dorsal spines are all of approximately the same length. The dorsal soft-rays are all longer than the spines and all, excepting the shorter ones at each end, are of approximately the same length. The two dorsal fins vary from distinctly to barely separated. The caudal is bluntly emarginate.

The first anal spine is much thicker than the second, which is similar to the dorsal spines. The anal soft-rays are all approximately twice as long as the spines.

The bluntly pointed pelvics extend two thirds of the distance to the anus; the rounded pectorals, three fifths of the distance to the anus.

Scales cover the body except the anterior part of the belly and most of the pectoral base (there are some embedded scales on the ventral part of the pectoral base). The row of enlarged and strongly ctenoid scales along the midline of the belly of males, separated from normal scales by a series of intermediate scales, is most pronounced during the breeding season. In females and immature males these scales are less modified. The dorsals, anal, pelvics, pectorals, and the distal two thirds of the caudal are naked. Several parts of the head are scaled: the opercle is covered with imbricate scales; scattered embedded scales are present on the side of the head above the opercle; the cheek has imbricate scales above a line between the lower edge of the pupil and the upper pectoral ray; and embedded scales occupy the lower cheek.

The color pattern of *Hadropterus scierus* in general and of *H. s. apristis* in particular exhibits relatively little sexual dimorphism or seasonal variation. The ground color is olive, with seven distinct rectangular black blocks along the midline (an additional indistinct one above the pectoral base and at the caudal base). The blocks are connected to eight indistinct gray saddles. The blocks are separated by an equal distance of ground color in females but are almost connected in males. Three dark spots are on the caudal base: the upper almost meets its opposite number across the procurrent rays; the middle is at the end of the lateral line; and the lower is about midway between the middle spot and the ventral surface. The middle and lower spots are often fused to the last body block, forming its darker posterior border. On the head an anterior continuation of the series of blocks forms an irregular line through the eye. The proximal half of the spinous dorsal is black in males and gray in females, and depending upon the season the distal part is yellow to orange in males and clear to yellow in females. The other median fins have gray marks on the rays that in combination form irregular bars. The paired fins vary from gray in breeding season to clear in other seasons.

Comparisons.—This and the other subspecies of *H. scierus* can be distinguished easily from their near relatives in Texas. *Hadropterus maculatus* has a longer upper jaw (nine percent of the standard length as compared with eight percent or less in *H. scierus*), no scales on the nape (extralimital *maculatus* may have the nape scaled), and a distinct roundish median black spot at the base of the caudal. *Hadropterus* (*Imostoma*) *shumardi*? has fewer scales (around 50 instead of 60 or more), a narrow frenum, and elongate tuberculate anal rays in the mature male.

The major part of the diagnosis of *H. scierus apristis* would fit not only this subspecies, but also the other races of *H. scierus*. The distinctions are brought out in the following comparison and in the tabulations discussed under racial variation. The most significant and consistent difference lies in the number of preopercular serrae (left side only counted). The counts for *apristis* are approached only by those of the Arkansas River specimens (fig. 1), among the series investigated. Although the rays of the median fins of *apristis* are shorter on the average than those of most other populations, the rays of the paired fins usually are longer. The dark rectangular blocks on the side are longer and narrower than in other races. The narrow bars that connect the dark blocks in typical *scierus* are reduced or absent in *apristis*.

Range, habitat, and abundance.—Hadropterus scierus apristis occupies all suitable habitats in the San Marcos and Comal rivers, except those around the spring heads. It has also been collected in the Guadalupe River, but no specimens have been taken west of Kerrville, despite the very extensive collecting reported on by Hubbs, Kuehne, and Ball (1953). It has never been taken in any tributary to the rivers named above. There are no specimens in the few collections available from the San Antonio River System.

The Guadalupe dusky darter is a riffle fish. It is most commonly found under or around ten- to twelve-inch boulders in the main current. It appears to prefer moderately turbid water, for it is absent from the clearest waters tributary to the Guadalupe, namely the spring heads and main river west of Kerrville, and it has been found most commonly east of the Balcones Escarpment, where water turbidity is high. It has never been obtained from a stream that is not permanent.

MATERIAL EXAMINED

The material on which this paper is based is deposited in the University of Michigan Museum of Zoology (UMMZ), United States National Museum (USNM), Chicago Natural History Museum (CNHM), Oklahoma Agriculture and Mechanical College (OAM), Texas Agriculture and Mechanical College (TAMC), Stanford Natural History Museum (SU), and Texas Natural History Collection (TNHC).

The holotype (UMMZ 162377) and 41 paratypes (UMMZ 162378) were collected by Kenneth C. Jurgens and Robert C. MacEwan from the San Marcos River, one half mile southeast of Prairie Lea, Caldwell County, Texas, on February 11, 1950. Eighty-nine other specimens from the San Marcos River are designated as paratypes (UMMZ 162374, USNM 160628 and 160629, CNHM 46195, OAM 4707, TAMC 1F-13-g-4 and 1F-13-g-10, SU 17311, and TNHC 90 and 501). Two specimens from the Guadalupe River (TNHC 162 and 314) are also designated as paratypes. The type series comprises the San Marcos sample in the racial discussion. Seventy-one specimens from the upper Guadalupe River are not designated as paratypes because they differ in minor ways from the San Marcos individuals. These specimens make up the Hill Guadalupe sample.

Comparative material is listed according to the groupings utilized in the racial study: Colorado River System, Texas, 12 specimens (UMMZ 129788 and 162376, USNM 160627, OAM 4702, and TNHC 1087, 1122, and 1416); Brazos River System, Texas, 49 (UMMZ 110528, 129776, 129895, and 162373, and TNHC 694 and 1024); San Jacinto River System, Texas, 57 (UMMZ 131158, USNM 160616, and TNHC 693 and 1583); Trinity River System, Texas, 58 (UMMZ 162371, CNHM 46196, SU 17313, and OAM 4703); Neches River System, Texas, 22 (UMMZ 162370, USNM 160615, CNHM 46195, OAM 4706, TAMC 1F-13-g-11, SU 17312, and TNHC 383 and 557); Sabine River System, Texas, 39 (UMMZ 162375, USNM 160614, OAM 4705, and TNHC 1521 and 1536); lower Red River System, Texas and Oklahoma, 12 (OAM 4686 and TNHC 1418 and 1458); upper Red River System, Oklahoma, 32 (OAM 1495, 1624, 2900, and 4103); Little River, Oklahoma, 50 (OAM 2878); Arkansas River System, Oklahoma and Arkansas, 26 (OAM 999, 1180, 1230, and 4546); and Wabash River System, Indiana, 19 (UMMZ 81345, 81386, and 113552).

RACIAL ANALYSIS OF HADROPTERUS SCIERUS IN TEXAS AND OKLAHOMA

Many of the proportional measurements used vary with specimen size. Each character was checked for such variation. For the two proportions used

that show marked age changes, the pectoral fin length and the upper jaw length, a constant was calculated that makes all the data approximately comparable (see legends for figs. 5, 7).

The specimens utilized in this study were collected at many localities (fig. 2). To increase the statistical validity, the collections are grouped according to stream system and, when adequate numbers of specimens are available (for the Guadalupe and the Red systems) they are grouped within a given system in subdivisions having the same general ecologic conditions. Some variation undoubtedly is obscured by grouping the collections, but this seems preferable to having too many very small samples and is justifiable on other grounds. The San Marcos, Sabine, and Wabash samples include specimens from, or near, the type localities of the three nominal subspecies, *apristis* Hubbs and Hubbs, *serrula* Jordan and Gilbert, and *scierus* Swain.

When the samples are compared graphically, three major groupings by stream systems appear: (1) Hill Guadalupe and San Marcos (*apristis* Hubbs and Hubbs); (2) Colorado, Brazos, San Jacinto, Trinity, Neches, Sabine, Lower Red, Upper Red, and Little; and (3) Wabash. In several respects the fish from the Arkansas River System are intermediate between those of morphologic types (2) and (3); in other ways they are somewhat distinctive. The Lower Red, Upper Red, and Little samples, although considered as type two, approach type three in several characters.

The *apristis* specimens have the fewest preopercular serrae (fig. 1). The lower limits of the standard deviation bars of all other samples except that from the Arkansas River System are separated from the upper limits of the standard deviation bars for *apristis* by a distance greater than one standard deviation. Although the Arkansas sample approaches *apristis* in number of preocular serrae, it is obviously distinct. There is no overlap between its standard deviation bar and that of either *apristis* sample in the caudal peduncle depth (fig. 3), caudal peduncle length (fig. 4), and pectoral length (fig. 5).

The greater length of the *apristis* pectoral fin is of statistical significance when compared with that of all other samples (fig. 5), though subspecific separation could not be based on this distinction alone.

The number of lateral-line scales of the Guadalupe dusky darter is statistically greater than that of any other population except that from the

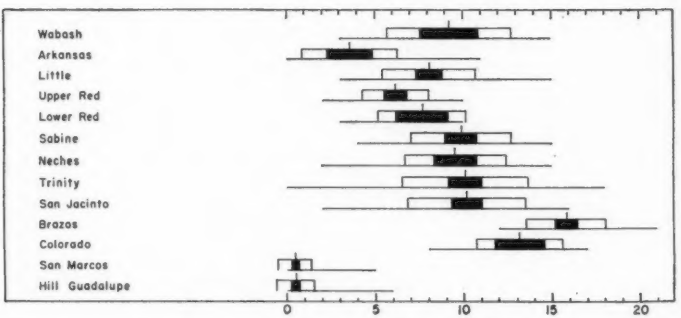


Fig. 1.—Number of preopercular serrae (left side only)

Little River (fig. 6), but again the overlap in counts is too wide for subspecific differentiation on this account.

It is possible that *apristis* may be specifically distinct from *scierus*. In distribution it is extreme (toward the southwest) and it is completely separated from the nearest population of other forms. The waters of the Colorado and

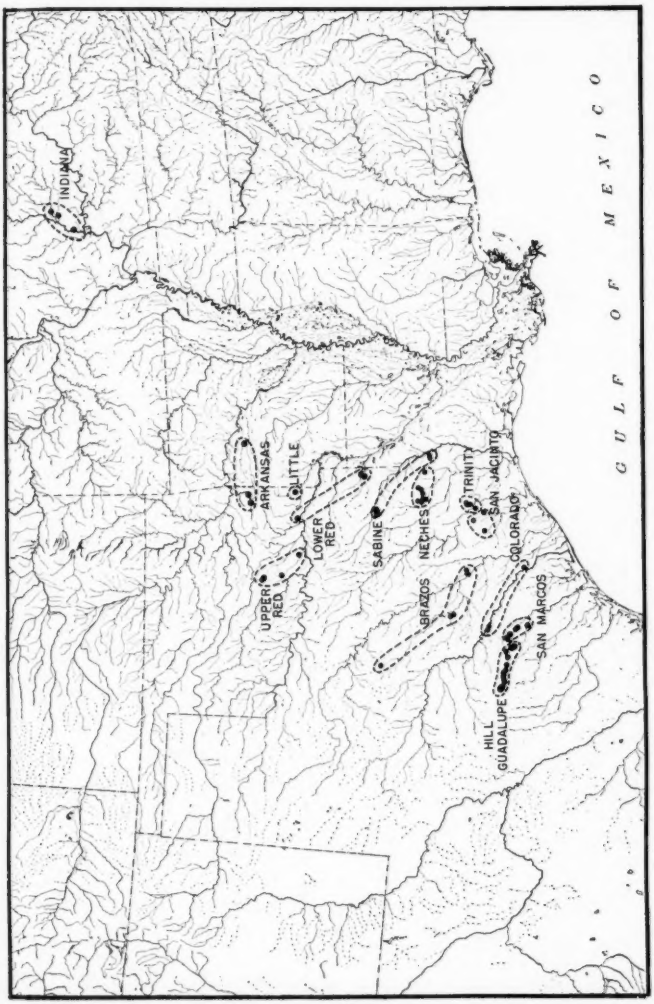


Fig. 2—Map of collection localities

Guadalupe rivers empty into the saline coastal water of Texas 50 miles apart. The fact that the populations geographically closest to the Guadalupe River often differ greatly morphologically from those of the Guadalupe River indicates that these populations may be specifically differentiated. The difference is especially notable in the number of preopercular serrae (fig. 1), in which character there appears to be no overlap between the two stocks. In fact the

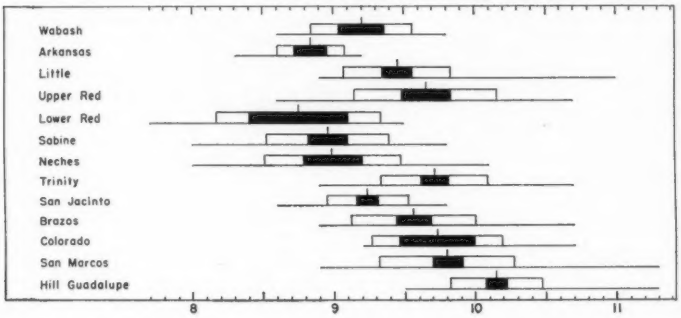


Fig. 3.—Caudal peduncle depth in hundredths of standard length

series of races from the Arkansas River System to the stream systems just north of the Guadalupe appears to be characterized by a cline that leads away from *apristis*. The Brazos and Colorado samples have the serrae better developed than in any other race of *scierus*, whereas *apristis* has the serrae least developed. The Colorado sample likewise contrasts with the *apristis* samples in pectoral length (fig. 5). Because of the considerable overlap in diagnostic characters when all races are considered, however, the Guadalupe race is considered to be only subspecifically distinct. The question of specific or subspecific rank may be solved when proposed fertility tests have been completed.

The specimens of morphologic type three (typical *scierus*) have the shortest upper jaw (fig. 7). When the tabular data are compared, the standard deviation of the Wabash sample overlaps only with that of the specimens from the Arkansas River system, which in this respect are intermediate.

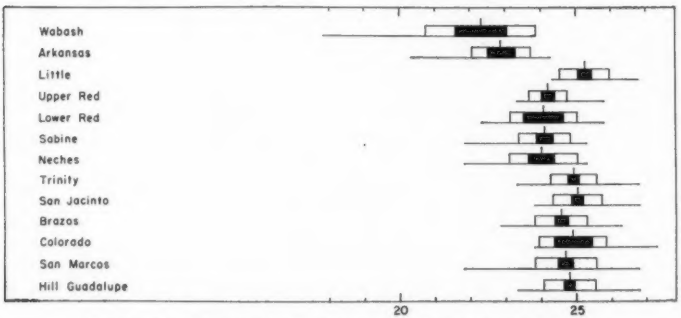


Fig. 4.—Caudal peduncle length in hundredths of standard length

The average snout length of the Wabash sample is the shortest of any studied (fig. 8). This distinction is statistically reliable for the comparisons of the Wabash race with all other samples except the three northernmost (Arkansas, Little, and Upper Red). The fishes from these three localities show definite type three characteristics, *i. e.*, each can be separated statistically (though not subspecifically) from the nine longer-snouted southern samples.

The Wabash specimens have the shortest pectoral fins (fig. 5). When the proportional data are plotted, the standard deviation bar of the Wabash sample overlaps those of the Arkansas, Little, Lower Red, Colorado, Upper Red, and Trinity samples. The overlap with the latter two is very slight, and their average pectoral length approximates that of the non-overlapping samples. The Colorado River System sample is small (twelve specimens) and as it is from

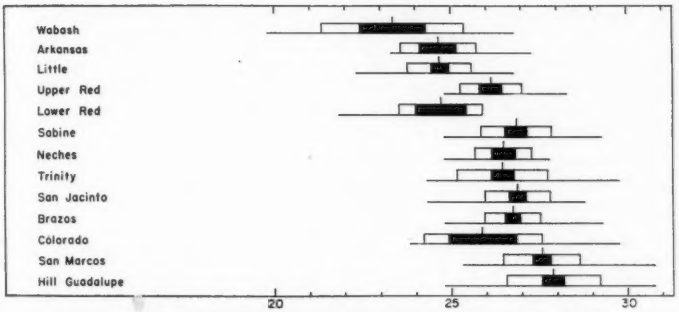


Fig. 5.—Pectoral length in hundredths of standard length + 0.07% per mm. of standard length

a river that is far from Indiana, its similarity to the Indiana specimens in this character may be attributed to sampling error or to independent modification. The three remaining samples (Arkansas, Little, and Lower Red) are intermediate in this respect.

The caudal peduncle of the Wabash sample is the shortest on the average of any studied (fig. 4). This character provides a statistically valid distinc-

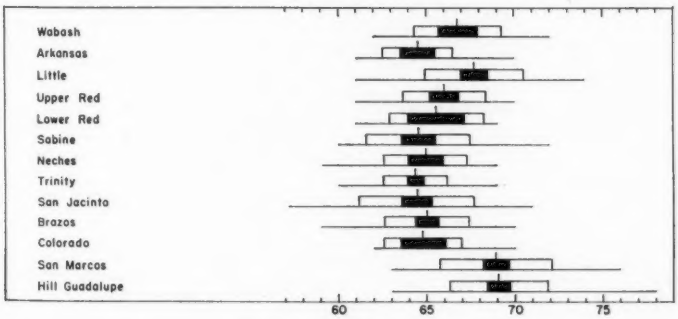


Fig. 6.—Lateral-line scales

tion in comparisons with all other samples except that from the Arkansas River. There is no overlap of the standard deviation bars of either the Wabash or Arkansas samples with those of the Little, Trinity, San Jacinto, Colorado, and Hill Guadalupe samples. The most extreme separation is with that of the Little sample.

Although the data just discussed suggest that morphologic types two and three are subspecifically distinct, I have not resurrected Jordan and Gilbert's name *serrula* for the southern type, because only one population of the northern type (*scierus* Swain) was studied. If analysis of other northern samples confirms the differences pointed out in this paper, the name *serrula* may be resurrected for a southwestern subspecies.

The characters used by Jordan and Gilbert (1886) to separate *serrula* from typical *scierus* prove inadequate for subspecific separation, as Hubbs and Black (1954) have noted. Jordan and Gilbert emphasized the smaller scales—68 to 71 in the lateral-line, as compared to 64 to 66 for Indiana specimens. My specimens from near the type locality of *serrula*, however, average 63.5 in scale count and near-topotypes of *scierus* average 65.6. Jordan and Gilbert's counts were either based on a small sample or on their San Marcos and New Braunfels specimens of *apristis*, which has smaller scales. The lighter color attributed to *serrula* refers to a character that varies with the clarity of the water and with season, sex, and preservation. The supposed difference in the squamation of the breast seems invalid, and this character varies with sex and season. The supposedly more distinct preopercular serrae apply only to the Brazos and Colorado samples, and the number of serrae in the Colorado basin sample overlaps the others too widely to be the chief subspecific character.

As is often true when more than one character distinguishes geographic races, the zone of intergradation between peripheral forms does not occur in the same region for all characters. The area of intermediacy in respect to the upper jaw length (fig. 7) and caudal peduncle length (fig. 4) is in the Arkansas River System. In snout length the line of intermediacy extends from Lake Texoma to Fort Smith, Arkansas. In pectoral length there is a somewhat similar broad area of intermediacy.

There are minor, probably racial, differences between the two samples of

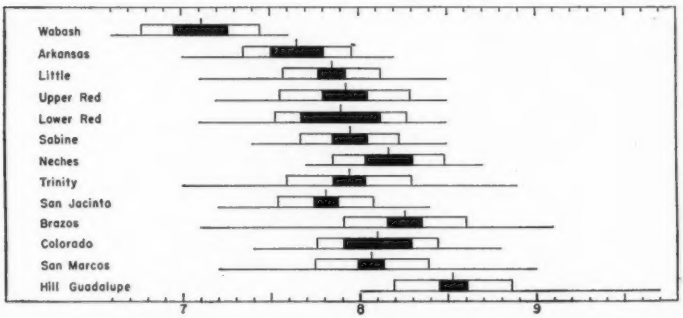


Fig. 7.—Upper jaw length in hundredths of standard length + 0.02% per mm. of standard length

morphologic type one (*apristis*) and among the several samples of type two.

The two samples of *apristis* show little or no statistically significant difference in five of seven characters presented, but exhibit statistically reliable differences in caudal peduncle depth (fig. 3) and in upper jaw length (fig. 7). The overlap, however, as measured by the standard deviation bars, is far too great to warrant subspecific separation. In the three characters shown in figs. 1, 5, and 6 the two samples of *apristis* agree closely and together contrast with all other samples.

In general, the samples of type two most closely approximated geographically (fig. 2) tend to be the most similar morphologically. The Colorado and Brazos samples are very similar, differing assuredly only in the number of preopercular serrae (fig. 1), in which respects both samples exceed any other.

The San Jacinto and Trinity samples closely resemble each other morphologically. The only reliable difference indicated is in the caudal peduncle depth (fig. 3), and the overlap in this respect is far too great for subspecific distinction. In only one character (fig. 3) are these two samples more similar to the Colorado and Brazos collections than to those from the Neches and Sabine.

The Neches and Sabine samples are especially similar, though there is an apparently trustworthy difference in the average length of the upper jaw (fig. 7). In most respects these samples closely resemble those from the adjacent Trinity and San Jacinto rivers to the southwest. Taken together, the Neches and Sabine samples resemble the Lower Red sample in all characters studied except the pectoral length (fig. 5), in which the Lower Red fish approach morphologic type three (typical *scierus*).

The Lower and Upper Red samples differ significantly, though not on the subspecies level, in three proportions (figs. 3, 5, 8). There are four more or less reliable differences between the Lower Red and Little samples (figs. 3, 4, 6, 8), but none of these distinctions would merit subspecies separation. The Lower Red fish are more similar to those of the Sabine and Neches than to the other samples of the same river system, namely those of the Upper Red and the Little. The Upper Red sample is not markedly similar to any other. It resembles the Trinity sample as closely as any other from Texas. The Upper

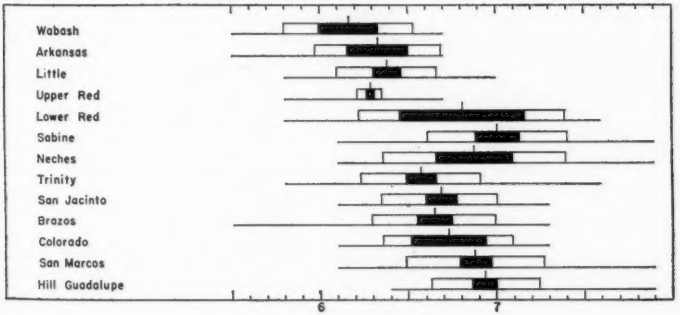


Fig. 8.—Snout length in hundredths of standard length

Red and Little samples differ in a number of respects. The snout length in the Upper Red sample is remarkably constant (fig. 8).

Each of the nine samples of morphologic type two can be separated on apparently reliable grounds from each other one by at least one character, and many pairs show little or no overlap between the standard deviation bars. It is possible that some other subspecific distinctions may be effected, but such action does not appear warranted from the present analysis.

SUMMARY

A darter endemic to the Guadalupe River System in Texas is described as a new subspecies of *Hadropterus scierus*. Its characters contrast with those of typical *H. scierus* and with those of the two other species of the same genus known to inhabit Texas waters, *H. shumardi?* and *H. maculatus*.

The two samples of *H. s. apristis* studied can be separated from each of 11 samples of *H. s. scierus* on apparently reliable grounds. Despite intersample variability, three morphological groupings occur: the two samples of *apristis*, nine samples from Texas and the Red River system in Oklahoma, and the Wabash River sample. The Arkansas River sample is intermediate between the latter two. Jordan and Gilbert's name *serrula* is not resurrected for the second morphologic grouping because the distinctions of the single northern sample may not hold when other samples are studied.

REFERENCES

BAUGHMAN, J. L. 1950—Random notes on Texas fishes. Part II. Texas J. of Sci. 2:242-263.

DICE, LEE R. AND HAROLD J. LERAAS 1936—A graphic method for comparing several sets of measurements. Contr. Lab. Vert. Gen. Univ. Mich. no. 5:1-3.

EVERMANN, BARTON W. AND WILLIAM C. KENDALL 1894—The fishes of Texas and the Rio Grande Basin, considered chiefly with reference to their geographic distribution. Bull. U. S. Fish Comm. 12:57-126, pls. 10-50.

FOWLER, HENRY W. 1945—A study of the fishes of the southern Piedmont and Coastal Plain. Monogr. Acad. Nat. Sci. Phila. 7:i-vii, 1-408, 73 pls.

HUBBS, CARL L. 1951—*Notropis amnis*, a new cyprinid fish of the Mississippi fauna, with two subspecies. Occ. Pap. Mus. Zool. Univ. Mich. no. 530: 30 pp., 1 pl.

——— AND JOHN D. BLACK 1954—Status and synonymy of the North American percid fish, *Hadropterus scierus scierus*. Amer. Midl. Nat. 52(1):201-210.

——— AND KELSHAW BONHAM 1951—New cyprinid fishes of the genus *Notropis* from Texas. Texas J. of Sci. 3:91-110.

——— AND CLARK HUBBS 1953—An improved graphical analysis and comparison of series of samples. Syst. Zool. 2:49-56+92.

——— AND KARL F. LAGLER 1947 (new printings, 1949 and 1952)—Fishes of the Great Lakes region. Bull. Cranbrook Inst. Sci. 27:xi+186.

——— AND ALFRED PERLMUTTER 1942—Biometric comparison of several samples, with particular reference to racial investigations. Amer. Nat. 76:582-592.

HUBBS, CLARK, ROBERT A. KUEHNE, AND JACK C. BALL 1953—The fishes of the upper Guadalupe River, Texas. Texas Jour. of Sci. 5:216-244.

JORDAN, DAVID S. AND BARTON W. EVERMANN 1896—A check-list of the fishes and fish-like vertebrates of North and Middle America. Rept. U. S. Comm. Fish. 21:207-584.

———, ——— AND HOWARD W. CLARK 1930—Check list of the fishes and fishlike vertebrates of North and Middle America north of the northern boundary of Venezuela and Colombia. Ibid. 1928(2):670 pp.

——— AND CHARLES H. GILBERT 1886—List of fishes collected in Arkansas, Indian Territory, and Texas, in September, 1884, with notes and descriptions. Proc. U. S. Nat. Mus. 9:1-26.

Notes on the Food of Three Species of Alberta Amphibians*

J. E. Moore and E. H. Strickland

University of Alberta, Edmonton

In view of the paucity of detailed information concerning the food of Alberta amphibians, it seems desirable to indicate the results of certain preliminary studies made at the University of Alberta in recent years. Our data on food habits were obtained by examination of the stomach contents of 92 specimens collected from the prairie region of southern Alberta. We were concerned with three of the common amphibians from this region, *Rana pipiens*, *Pseudacris nigrita*, and *Bufo hemiophrys*.

These studies have involved analyses of 20 stomachs from adult *R. pipiens*, 37 from adult *P. nigrita*, and 35 from *B. hemiophrys* of which 16 came from adults and 19 from juveniles. A summary of the determinations is given in table 1, in which, under each species, we have indicated the number of stomachs containing the various groups of invertebrates and also the numbers of invertebrate specimens represented. All identifications of the invertebrate material were by the junior author. It may be noted that many of the stomachs contained small amounts of vegetation and sand in addition to the animal matter reported.

Rana pipiens.—The specimens utilized in the investigation of this species were adults obtained as follows: 14 from High River collected August 25, 1951; 5 from Eagle Butte, August 5, 1951; and 1 from Lethbridge, July 23, 1951. The data indicate that leopard frogs in these localities feed mainly on arthropods and to a slight extent on earthworms, snails and slugs. Insects make up the major portion of the arthropod material while arachnids such as spiders and harvestmen are of lesser importance. Although a wide variety of insects serve as food, it seems apparent that Coleoptera, Orthoptera and Diptera constitute the mainstay of the diet.

The following notes** contain a detailed account of the items represented in the 20 stomachs: *Mollusca*—8 snails and 1 slug. *Annelida*—portions of 2 earthworms. *Arachnoidea*—11 spiders, 10 harvestmen, and 2 gamasid mites (probably taken incidentally). INSECTA: 150 specimens including: *Collembola*—single individual. *Orthoptera*—10 specimens comprising locusts (1 *Mestobregma kiowa*), grouse locusts, and crickets. *Odonata*—2 nymphs and 1 adult damselfly, 5 nymphs and 2 adult dragonflies (1 *Sympetrum* sp.). *Hemiptera*—1 pentatomid (*Aelia americana*), 2 coreids, 1 lygaeid (*Geocorus bullatus*), 2 mirids (*Stenodema trispinosum*), 3 gerrids (1 *Gerris remigis*), and 1 corixid. *Homoptera*—3 cercopids, 1 nymph and 9 adult cicadellids (1 *Cuerna costalis*), 3 fulgorids, and 1 psyllid. *Coleoptera*—5 carabids (1 *Harpalus* sp. and 1 *Carabus taedatus*),

* The material which formed the basis of this study was obtained during a zoological survey made in 1951 by the senior author with support from the General Research Fund of the University of Alberta.

** In these notes and also in those which follow, the beetles are listed according to the *Catalogue of the Coleoptera of America, North of Mexico* by C. W. Leng and the flies according to the arrangement proposed by C. H. Curran in *The Families and Genera of North American Diptera*.

5 larval and 2 adult *dytiscids*, 1 silphid (*Silpha ramosa*), 1 meloid (*Lytta* sp.), 1 coccinellid, 2 tenebrionids (*Eleodes opaca*), 1 chrysomelid, 4 curculionids (3 *Otiorhynchus sulcatus*), and 11 undetermined beetles of which 6 were aquatic larvae, 2 terrestrial larvae and 3 terrestrial adults. *Trichoptera*—2 caddis fly adults. *Lepidoptera*—5 moths (4 noctuids). *Diptera*—7 tipulids, 3 dolichopodids (1 *Sympycnus* sp.), 9 ephydrids, 4 muscids, 8 metopiids of which 6 were larval, 1 each of chironomid, bibionid, asilid, pipunculid, conopid, sepsid, agromyzid, tetanocerid, and tachinid, besides 2 larval and 2 adult Diptera undetermined. *Hymenoptera*—5 ichneumonids (1 *Ichneumon* sp.), 1 braconid, 3 sphecids (1 *Nysson* sp.), 8 formicids (3 *Formica fusca* and 1 *F. sanguinea*), 1 andrenid.

Pseudacris nigrita.—Of the 37 adults involved in the study of this species, 7 were collected at Alsask on June 24, 1951; 5 at Irricana July 5, 1951; 10 at Lethbridge July 23, 1951; 11 at Eagle Butte August 5, 1951; and 4 at Barons August 15, 1951. The food of this small species was exclusively arthropod in nature and consisted mainly of insects. In addition there were indications of a slight utilization of spiders, mites and centipedes. It is apparent that a wide variety of insects is consumed and that Diptera and Coleoptera form the main sources of food. As might be expected, this species appears to feed more on the smaller insects. Small flies are an important constituent of the diet while large insects such as the Odonata were not represented in the stomachs. The meloids appear to have been selected for their small size and were far below the average. The noctuids were, however, of the usual size for this family.

The details of the analyses are as follows: *Arachnoidea*—7 spiders and 8 Gamasid mites. *Chilopoda*—single individual. INSECTA: 310 specimens comprising: *Collembola*—1 individual. *Orthoptera*—1 cricket nymph and fragments of 2 grasshoppers. *Plecoptera*—2 nymphs. *Hemiptera*—5 coreids (1 nymph), 3 lygaeids (*Nysius ericae*), 1 mirid, 9 saldids, 1 gerrid nymph, 1 corixid, 2 nymphs and 5 adult undetermined. *Homoptera*—5 cercopids, 14 cicadellids, 1 fulgorid, 3 psyllids and 4 aphids. *Coleoptera*—14 carabids involving 1 larva, 6 adult *Bembidion* sp. and 7 unidentified adults, 1 silphid (larva of *Silpha* sp.), 6 staphylinids, 15 meloids (*Meloe* sp.), 2 elaterids, 2 nitidulids, 1 scarabaeid (*Aphodius* sp.), 5 chrysomelids, 5 curculionids, 4 aquatic and 3 terrestrial larvae undetermined. *Lepidoptera*—4 moths (2 noctuids) and 1 caterpillar. *Diptera*—21 tipuloids (13 winter gnats, 1 *Nephrotoma altissima*, 2 larvae and 5 adults unidentified), 17 Chironomids (12 *Chironomus plumosus*), 2 bibionids, 2 dolichopodids, 4 sepsids, 4 agromyzids, 8 chloropids, 26 ephydrids, 16 borborids (*Borborus equinus*), 10 muscids, 55 metopiids of which 37 were larvae, 1 specimen of each of the following: ceratopogonid, culicid (*Aedes* sp.), mycetophilid, undetermined nematoceran, lonchopterid, pipunculid, conopid, lauxaniid. *Hymenoptera*—2 ichneumonids (1 *Gelis* sp.), 1 braconid, 3 chalcids, 2 drinids, 1 sphecid and 9 formicids (1 *Camponotus* sp.).

Bufo hemiophrys, Adults.—Information on the food of adult Canadian toads came from a study of the stomachs from 16 specimens. Thirteen were collected at Alsask on June 24, 1951; 2 at Empress, July 12, 1951; and 1 at Irricana July 5, 1951. The material present in the stomachs was almost entirely of arthropod origin. Indeed, with the exception of one stomach which contained two snails, there was no indication of other invertebrates. Insects make up the bulk of the diet for the adult Canadian toad. Although a wide variety of insects are taken, the Coleoptera and Hymenoptera are most important as food items. The main families of beetles are carabids, silphids, chrysomelids and curculionids while the Hymenoptera are represented chiefly by various kinds of ants. Non-insect food consisted largely of spiders—in most stomachs there were remains of two or three small spiders. It would appear that the over-all picture of the food of *Bufo hemiophrys* adults is quite comparable to that determined by Smith and Bragg (Ecology 30:333-339, 1949) for four common species of toads in Oklahoma.

There follows a list of the arthropods recognized in our analyses: *Arachnoidea*—34 spiders and 1 large water mite. INSECTA: 1834 specimens representing 9 orders: *Orthoptera*—2 undetermined grasshoppers, 1 grouse locust (*Acrydium granulatum*), and 1 field cricket (*Gryllus assimilis*). *Ephemeroptera*—1 mayfly nymph. *Odonata*—1 damselfly nymph. *Hemiptera*—5 coreids, 1 lygaeid and 7 saldids. *Homoptera*—1 cicadellid (*Gypona* sp.) and 6 fulgorids. *Coleoptera*—58 carabids (6 *Carabus violaceus* and 3 larvae of *Calosoma* spp.), 1 omophronid (*Omophron americanum*), 13 dytiscids of which 12 were larvae, 1 hydrophilid, 52 silphids (35 adults and 7 larvae of *Silpha opaca* and 10 adult *S. ramosa*), 3 staphylinids, 3 meloids (2 *Epicauta* sp. and 1 *Lytta* sp.), 5 elaterids, 1 buprestid (*Agrillus* sp.), 2 dermestid larvae, 4 coccinellids, 12 scarabaeids (4 *Canthon* sp. and 1 larva of *Aphodius* sp.), 1 cerambycid, 48 chrysomelids (28 *Disonycha* sp., 2 *Gastroidea cyanea* and 1 *Donacea* sp.), 90 curculionids (63 *Otiorhynchus sulcatus*, 1 *Lixus* sp. and 1 *Lepyrus* sp.), 12 adults and 1 aquatic larva undetermined. *Lepidoptera*—2 caterpillars. *Diptera*—95 tipuloids (all were winter gnats which occurred in 3 stomachs only), 3 ceratopogonids, 1 specimen of each of the following: asilid, empid, dolichopodid, lonchopterid, sepsid, ephydrid, borborid, muscid, and 1 undetermined fly. *Hymenoptera*—1 tenthredinid larva, 10 ichneumonids (1 *Gelis* sp.), 1381 formicids (147 *Formica fusca*, 139 *F. sanguinea*, 1092 *Myrmica brevispinosa*, and 3 undetermined large black ants). All but three of the stomachs contained ants of some kind.

Bufo hemiophrys, Juveniles.—The stomachs used in the investigation of food taken by juvenile *B. hemiophrys* were obtained from 19 specimens (lengths 15 to 20 mm) collected at Empress on July 12, 1951. The food material consisted mainly of small beetles and flies with mites, springtails and hymenopterans being of secondary importance. Rove beetles supplemented by such Diptera as psychodids, chironomids, borborids, and ephydrids made up the bulk of the material. Juveniles apparently feed chiefly on the smaller representatives of the insects which are most available, with the exception of ants. The relative unimportance of ants in the diet of juveniles stands in marked contrast to the situation for adults.

The details of the stomach contents are as follows: *Arancida*—4 spiders. *Acarina*—14 water, 57 oribatid type, 73 manure-inhabiting type, and 10 gamasid mites. *Collembola*—46 specimens. *Thysanoptera*—9 specimens. *Hemiptera*—2 saldids. *Homoptera*—3 cicadellids, 1 fulgorid, 1 psyllid and 8 aphids. *Coleoptera*—16 carabids, 2 dytiscid larvae, 19 hydrophilids, 654 staphylinids, 1 histerid, 7 anthicids (*Notoxus* sp.), 8 scarabaeids (*Aphodius* spp.), 12 larval and 10 adult chrysomelids, 5 curculionids, 29 undetermined specimens including 2 aquatic larvae, 4 terrestrial larvae and 23 adults. *Diptera*—2 tipulid larvae, 40 chironomids (13 larvae, 2 pupae and 25 adults), 5 ceratopogonids, 79 psychodids, 3 dixid pupae, 5 cecidomyids, 2 sciarids, 2 mycetophilids, 1 bibionid, 2 stratiomyids (aquatic larvae), 1 dolichopodid, 1 syrphid larva, 10 sepsids, 1 lauxaniid, 5 agromyzids, 5 chloropids, 20 ephydrids, 31 borborids, 2 muscids, 2 metopiid larvae, 9 undetermined (4 larvae and 5 adults). *Hymenoptera*—2 ichneumonids, 7 braconids (1 *Chelonus* sp.), 4 chalcids, 14 serphids, 6 cynipids, 10 formicids (1 *Formica fusca*, 4 *F. sanguinea*, and 5 *Myrmica brevispinosa*).

Our observations indicate that adult Canadian toads feed mainly on ground-dwelling insects such as ground beetles, carrion beetles, and ants. Juvenile toads consume large numbers of ground insects (chiefly rove beetles) but, in addition, utilize substantial quantities of flying insects especially small flies. Adult leopard frogs and swamp tree frogs, on the other hand, take a greater proportion of flying insects. They also utilize ground-dwelling insects but appear to ignore such prevalent types as ants. Furthermore, the frogs, because of their more aquatic habits, feed on aquatic insects to a greater extent than do adult toads.

TABLE 1.—Stomach Contents of 20 *Rana pipiens*, 37 *Pseudacris nigrita*, 16 Adult and 19 Juvenile *Bufo hemiophrys*

	Rana pipiens		*Pseudacris nigrita*		*Bufo hemiophrys* Adults		*Bufo hemiophrys* Juveniles	
	No. stomachs containing designated invertebrates	No. of invertebrate specimens	No. stomachs containing designated invertebrates	No. of invertebrate specimens	No. stomachs containing designated invertebrates	No. of invertebrate specimens	No. stomachs containing designated invertebrates	No. of invertebrate specimens
Mollusca	5	9	....	....	1	2	....	....
Annelida	2	2	....	....	....	....	....	....
Arthropoda	20	173	37	326	16	1869	19	1262
Arachnoidea	14	23	9	15	13	35	18	158
Araneida	9	11	7	7	12	34	2	4
Phalangida	6	10	....	....	....	....	....	....
Acarina	2	2	4	8	1	1	18	154
Chilopoda	....	....	1	1	....	....	....	....
Insecta	20	150	37	310	16	1834	19	1104
Collembola	1	1	1	1	....	....	14	46
Orthoptera	7	10	3	3	3	4	....	....
Plecoptera	....	....	2	2	....	....	....	....
Ephemeroptera	....	....	....	....	1	1	....	....
Odonata	5	10	....	....	1	1	....	....
Thysanoptera	....	....	....	....	....	....	7	9
Hemiptera	7	10	15	23	5	13	2	2
Homoptera	13	17	15	27	5	7	8	13
Coleoptera	16	33	28	58	16	307	19	763
Trichoptera	2	2	....	....	....	....	....	....
Lepidoptera	5	5	5	5	2	2	....	....
Diptera	15	44	32	173	10	107	19	228
Hymenoptera	9	18	13	18	14	1392	18	43

Observations on the Life Cycles of Painted Turtles (Genus Chrysemys)

Fred R. Cagle
Tulane University, New Orleans, Louisiana

The members of the Genus *Chrysemys* provide excellent material for the study of the comparative life histories of turtles. The four subspecies occupy an area that includes the greater part of continental United States and Canada north to 51° N. Latitude. The limits of distribution of the three northern forms are well defined but the eastern and western limits of the range of *Chrysemys picta dorsalis* (Agassiz) are not known.

The differences in area of the ranges of the four forms is striking. *Chrysemys picta belli* (Gray) occurs from Washington and Oregon on the West southeastward to northern Mexico and northeastward to Missouri and Illinois and to 51° N. Latitude. *Chrysemys picta marginata* (Agassiz) occurs from the southern part of Ontario southwest through Michigan, eastern Illinois to northern Tennessee and northeastward to Pennsylvania, thence north through New York and extreme western Quebec to 51° N. Latitude. *Chrysemys picta picta* (Schneider) occurs from Quebec, Nova Scotia and New Brunswick southward through Maine, Vermont and New Hampshire along the Atlantic Coast to Florida. *Chrysemys picta dorsalis* (Agassiz) occupies the Gulf Coast plain from extreme southern Illinois south to the Gulf. The westward and eastward limits of its range are poorly defined, but the form apparently does not occur in eastern Kentucky, Tennessee or Alabama or western Missouri, Arkansas or eastern Texas. A population in southern Alabama and western Florida may represent a fifth subspecies.

Intermediates between each form occur at the junctures of the ranges. Individuals intermediate between *Chrysemys picta belli* and *Chrysemys picta marginata* are found in a zone about 200 miles in width extending from southern Illinois north to Ontario. The valley of the Hudson River and the areas of Massachusetts, Rhode Island, Connecticut, Delaware, eastern Pennsylvania and Maryland support a population intermediate between *C. picta marginata* and *C. picta picta*. A population that reflects the characters of *C. picta belli*, *C. picta marginata* and *C. picta dorsalis* occurs in extreme southern Illinois.

A program of research was undertaken in 1937 aimed at exploring possible ecological differences between populations occurring in northern Michigan, southern Illinois and Tennessee. The studies have since been extended to the Gulf Coast.

The bulk of our knowledge of these turtles is contained in short notes, brief discussions in regional reports and the work of experimental biologists. There have been no intensive studies of the bionomics of any one group.

Acknowledgement.—I am indebted to many present and former students for their enthusiastic participation in field work. Particularly valuable have been the contributions of Mr. Philip Smith, Mr. Lendell Cockrum, Mr. A. H. Chaney and Mr. Leslie Ellis. A series of turtles essential to the evaluation of growth determinations was loaned by Dr. Norman Hartweg of the Museum of Zoology, University of Michigan.

Habitat.—These turtles generally occupy static waters such as lake borders, ponds, and quiet creeks. They are but rarely found in streams with current

and then they occur only in sheltered inlets. Collecting in Louisiana has produced this turtle only from bayous, ponds, and small, shallow-water lakes. The southern form certainly avoids larger water bodies and streams much more than do the northern forms.

Procedure.—These investigations were directed toward obtaining information on the populations occurring in northern Michigan, southern Illinois, western Tennessee and the Gulf Coast. Populations were sampled by hand collecting, trot lining, dip netting and trapping. Individuals were marked, measured, dissected and data recorded as was described in a previous report on *Pseudemys scripta troosti* (Cagle, 1950).

Systematic turtle collecting was done in Illinois at varying intervals from 1937 to 1943 and intensive collecting in Louisiana from 1946 to 1951.

Comparative populations.—The major components of the data to be recorded here were the result of the study of 593 individuals of the two subspecies *C. p. marginata* and *C. p. dorsalis*. Of these, 246 were from Michigan (2 localities); 227 from Illinois (4 localities); Tennessee 66 (one locality); and Louisiana 54 (statewide). Other individuals were observed but could not be examined carefully in neither the field nor laboratory.

The Michigan turtles were collected from the shallow waters bordering Douglas Lake in the northern tip of the lower peninsula, from Nigger Creek about 6 miles from Douglas Lake, and from the vicinity of Ann Arbor. Collections were made by both trapping and hand collecting but most of the turtles were taken by hand. These turtles were uncommon in Douglas Lake but were abundant in the creek, where 240 were taken.

These turtles are much more abundant in southern Illinois than in any of the other areas studied. Most of the ponds, lakes, and small streams support a population (Cagle, 1942). The size distribution of Illinois and Michigan individuals have a a pattern similar to that reported for other reptile populations. Females and males of a size group near that in which sexual maturity is attained are typically the most abundant and but few larger individuals occur (fig. 1). This concentration near the size of attainment of sexual maturity is a reflection of the great slowing of growth at that time. These frequency distributions reflect another attribute of most reptile populations, the great abundance of individuals in the smaller size groups as contrasted to those in the size range just prior to those in which individuals become sexually mature.

The abundance of these turtles in Illinois and Michigan is in striking contrast to Louisiana where the members of this genus are difficult to find in any water body and have not been observed to be abundant in any area. The explanation for this extreme difference in abundance is not apparent but may reside in the more successful competition of the pseudemyid turtles in the southern area (Cagle and Chaney, 1950).

The slow-moving waters of southern Louisiana would appear to provide an ideal habitat for this turtle. The range and scattered occurrence suggest that the southern form has more delimited habitat requirements than the other subspecies. The records of Louisiana turtle-buyers for the novelty market provide one of the best indices to its relative abundance. These buyers purchase juveniles of the genera *Chrysemys*, *Pseudemys* and *Graptemys* in large quantity. Mr. Horace Whitten of the Waubun Laboratory, Shriever,

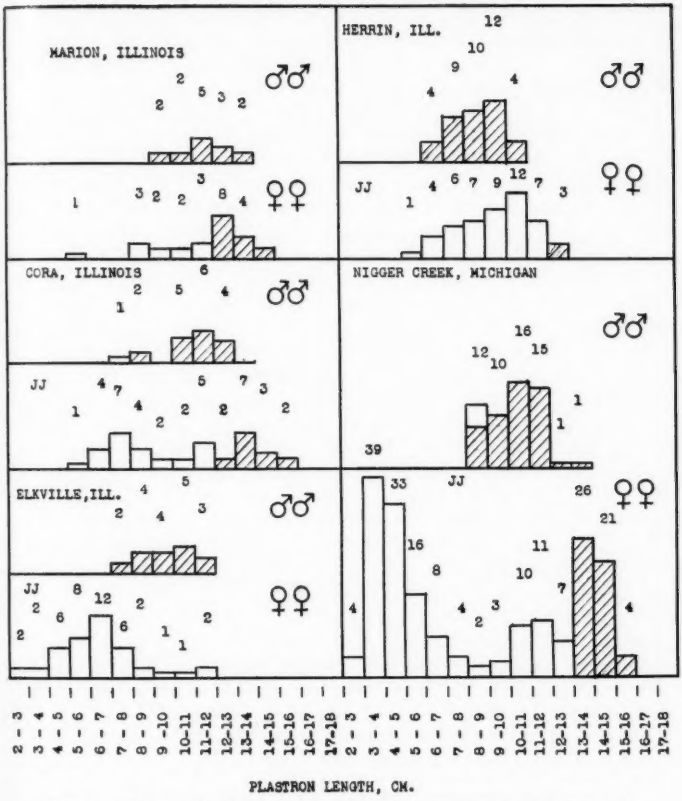


Fig. 1.—The size distribution of turtles in five populations. Columns with diagonal lines indicate sexually mature individuals

Louisiana, interviewed two of these buyers. He reports that of the total purchases by one dealer, Mr. Thomas Haydel, 50 percent were *Pseudemys* (*P. scripta troosti* and *P. floridana mobilensis*), 30 percent *Graptemys* (*pseudogeographica* subspp.) and 20 percent *Chrysemys*. The great majority of the painted turtles came from the vicinity of Grosse Tete, Louisiana. Another buyer, Mr. Robichaux of Raceland, Louisiana, who purchases turtles from the area of Houma, Paradis and Bayou Gauche, Louisiana, reported that he received no *Graptemys*, about 2 percent *Chrysemys* and 98 percent *Pseudemys*. These differences in percent of occurrence reflect the different areas in which the buyers operate. Mr. Haydel purchases many turtles from central Louisiana where map turtles (locally called gray-backs) are very abundant.

Nest construction.—The construction of nests by painted turtles has been

described in general terms repeatedly (Newman, 1906; Thacker, 1924; Cahn, 1937; Cagle, 1937). The observations made during this invesigation contribute no new facts to the available knowledge and demonstrate no differences between the subspecies. Many questions relative to the turtles nesting behavior do, however, remain unanswered. Is there any preferred nesting site in terms of distance from water, type of soil, humidity, exposure to sunlight? How is the choice or availability of nesting site related to mortality of hatchlings? What are the primary factors of mortality resulting from choice of nesting site; deviations in losses to predation, trapping in the nest, freezing, desiccation, or damage during embryonic development?

Eggs.—Although it is established that the eggs of female reptiles of a local population may be extremely variable in size, the possibility of significant differences in egg size between populations must be considered. As reptile eggs increase much in size and weight during development (Cunningham and Huene, 1938; Lynn and von Brand, 1945) comparison can be drawn only between eggs measured immediately after deposition or dissection from the oviducts or those retained under known conditions of temperature and humidity. Many investigators have reported the dimensions of eggs removed from nests (Cahn, 1937:144; Cagle, 1937:93; Breckenridge, 1944:182), but these measurements cannot be evaluated as the age of the egg or conditions under which it was retained is not given. The publication of descriptions of reptile eggs without data essential to the utilization of the information by other workers is fruitless. The suggestion of Lynn and von Brand (*op. cit.*) that because of irregularities in shape volume, a better criterion than lengths and widths, should be considered by students of reptile natural history.

Ninety-five eggs removed from Illinois individuals were 2.21-3.90 cm. (mean 3.09) in length, 1.60-1.96 cm. (mean 1.73) in diameter and 5.5-7.6 grams (mean 6.15) in weight. Seventy-one eggs dissected from Tennessee turtles were 2.81-3.60 cm. (mean 3.06) in length, 1.24-2.03 cm. (mean 1.83 cm. in diameter) and 4.9-9.3 grams (mean 5.0) in weight. There is no significant difference between these measurements.

Natality.—Information required in the estimation of natality is not available for any reptile population. Such data is essential for comparative population studies but requires the massing of information on attainment of maturity, period of reproductive capability, number of clutches per season, the effect of the sex ratio on fertilization rate, and the population density. Although data adequate for definition of all these factors is not available for these turtles, an attempt is made to gain an approximation of the annual reproductive potential and to explore the possibility of north-south differences between populations.

Each female may produce 2-11 eggs per brood and two broods each season. In 48 egg-containing southern Illinois turtles the number of eggs varied from 3 to 8 (mean 6.3). In 13 egg-containing females from Reelfoot Lake, Tennessee collected June 28, 1940 the number varied from 3 to 6 eggs (mean 4.3). In 12 females from northern Michigan the number of eggs varied from 2 to 7 (mean 4.7). Only oviducal eggs are included in these counts and they are therefore only suggestive of the real number per clutch as in some individuals eggs may have been deposited or ovulation incomplete. Ovulation points were not counted. Females collected in May, June and early July and con-

taining oviducal eggs usually had large ovocytes still present. Six such Illinois females collected May 21-June 15 contained respectively: plastron length 11.8 cm., 4 oviducal eggs, 4 ovocytes (1.3-2.2 cm. in diameter); Pl. 12.01 cm., 4, 5 (1.0-1.8 cm.); 13.5 cm., 3, 13 (1.0-1.1 cm.); 12.0 cm., 5, 6 (1.3-1.8 cm.) 12.1 cm., 5, 8 (1.0-1.4 cm.); 13.3, 6, 7 (1.0-1.6 cm). An Illinois female collected in late July contained 5 oviducal eggs but only 3 ovocytes 1.0 cm. in diameter.

It is assumed that these enlarged ovocytes represent second or third broods that would be deposited in one season. Agassiz (1857:490) believed that this turtle deposited only one clutch of eggs each year and interpreted the size groups of ovocytes to represent clutches that would be deposited in other years. Too, he suggested that these groups of ovocytes were associated with the need for two copulations each year for four successive years to stimulate ovulation. These interpretations have been discussed elsewhere (Cagle, 1950). The total annual reproductive capacity for females may thus be in excess of 39 eggs (maximum of 13 per brood; maximum of 3 broods per season) but probably averages approximately 15 eggs per season.

Counts of eggs in nests were made in Illinois, Tennessee and Louisiana. Seven Illinois nests contained 3, 6, 6, 4, 5, 7, 5 eggs respectively. Two Michigan nests contained 5 eggs each; one Louisiana nest contained 6 eggs.

The following constitute acceptable records: *C. p. marginata,* Cahn (1937:137) Illinois—4-10 eggs; *C. p. belli,* Cahn (1937:143) Illinois—4-10 eggs; Blanchard (1921:24) Iowa—(10 nests) 5-13 eggs; *C. p. dorsalis,* Cagle (1937:88) Tennessee—(8 nests) 4-10 eggs; Marchand (1942:71) Tennessee—(8 females) 3-6 eggs; Lynn and von Brand (1945) Maryland—(females) 2-8 eggs.

Although several authors have stated that these turtles may deposit as many as 10 eggs, few records based on dissections or field observations are available to verify such statements. Thus Cahn (1937) reported a maximum of 10 eggs for *C. p. marginata, C. p. belli* and *C. p. picta* but did not in either instance support his data. Smith (1950:147) indicates that females of *C. p. belli* may deposit as many as 15 eggs. This statement is apparently based on one made by Pope (1939:196) that a record number of 15 eggs for this turtle of this subspecies was based on a count from Iowa. I can find no verification for these reports. Blanchard (1921:24) reported that 10 Iowa nests contained 5-13 eggs. This record is the only one available that indicates more than 11 eggs for any of the three subspecies. Nest counts may, however, be unreliable. Repeated observations of 2 females depositing clutches in the same or connecting nests indicate caution in use of such data.

There is no correlation between the number of eggs and size of female but it is emphasized that these egg counts may be incomplete.

Hatchlings.—The estimation of growth rate requires that the size of the turtle at hatching be known. The young turtle changes much in appearance during the first few days after emergence from the egg and does not assume the size and proportions of the juvenile until after approximately 10 days. These changes are not here interpreted as growth.

Two individuals that hatched July 14, 1947 from eggs incubated at 30°C. were measured daily for 10 days. Both increased in carapace length (0.8 mm. mm. and 0.9 mm.); carapace width (1.6 mm. and 2.8 mm.), plastron length

(0.2 and 0.3 mm.) and plastron width (0.1 and 0.8 mm.) but decreased in height (1.8 and 2.4 mm.) and weight (0.6 and 0.5 grams). On the 10th day the hatchlings were respectively: Carapace length 26.1, 27.4 mm., carapace width 24.2 mm., 25.2 mm., plastron length 24.4 mm., 25.5 mm., plastron width 15.1 mm., 16.0 mm., height 11.6 mm., 13.2 mm.

The plastron lengths of 30 Louisiana hatchlings collected February 13, 1950 were 2.37-3.07, mean 2.67 cm.; the plastron lengths of 10 Illinois turtles collected April to July but not having grown were 2.40-2.60, mean 2.46 cm. Eleven Michigan hatchlings were 1.92-2.98, mean 2.46 cm. in length.

The hatchling retains a large yolk mass that is rapidly utilized during the first few months. The mass of the yolk retained at hatching is exceedingly variable and is apparently related to the temperature levels and changes during incubation. It is not demonstrated that this yolk is important to the survival of the turtle.

The turtle may spend the first winter in the nest (Nichols, 1933; Hartweg, 1944). Other authors have suggested that overwintering of young or "forced hibernation" of embryos may occur (Newman, 1906:144; Nichols, 1933:41; Breckenridge, 1944:182; Finneran, 1948:126). The frequent spring collecting of hatchlings with the egg tooth and unclosed umbilical scars in Illinois, Tennessee and Louisiana indicates that overwintering in the nest is common. This apparently may result from low temperatures or hardened earth preventing escape from the nest cavity.

Growth.—The estimate of growth during the first year is based on release-recovery data and calculated increments from growth ring measurements (Cagle, 1946). Growth ring measurements cannot be utilized unless it is demonstrated that the calculated growth increments are equivalent to known growth increments. The validity of growth increments calculated from ring measurements has been demonstrated for *Pseudemys scripta troosti* (Cagle, 1946, 1950).

There was no significant difference between the length of the smallest ring present on the abdominal plate of 25 Illinois turtles 1-3 seasons of age and the length of the abdominal plate of 25 Illinois hatchlings that had not grown. The ring measurements were 4.6-6.6 mm., mean $5.36 \pm .058$; abdominal plate measurements were 3.3-6.6 mm., mean $5.27 \pm .055$; the difference of the means divided by the standard error is 1.40 and is not significant.

Similarly there was no significant difference between the plastron lengths calculated from abdominal plate lengths and actual plastron lengths of hatchlings from Michigan, Illinois, or Louisiana. Too, there was no significant difference between abdominal plate lengths of hatchlings and lengths of the first growth rings on individuals older than one year.

It is concluded that growth calculations made for the first season from measurements of rings on the abdominal plate are valid. As no allometric growth has been demonstrated in the plastral plates and a rough test gives no indication that such growth occurs, it will be assumed that ring measurements provide useful approximations of seasonal growth in plastron length.

Individuals do not initiate growth until they have escaped from the nest and entered the water. Some of the Illinois hatchlings collected in April, May and July had egg-tooth scars and had not grown. One individual collected July 16, 1940 had an open umbilical scar, an egg tooth and the flexible

carapace typical of a recently hatched turtle. It is thus not possible to establish the precise time for the initiation of growth during the first season other than by intensive study in specific water bodies.

An estimate of the potential growth rate during the first season may be gained from measurements on those individuals having completed one season of growth. Three Illinois turtles collected in early April at the beginning of their second growing season had increased in plastron length 1.63 cm., 1.00 cm., and 0.21 cm. respectively. Fourteen Louisiana (Vaucherie, Paradis, La.) turtles studied after completion of the first growing season had increased 0.34 to 2.97, mean 1.08 cm. The turtle having the greatest increment (2.97 cm.) was a juvenile male that would probably become sexually mature in its next growing season. Twelve Michigan turtles (central and northern tip of lower peninsula) had increased 1.45-2.67, mean 1.91 cm. in plastron length during their first season.

The rapidity of growth during the first year of life is further stressed by the growth increments of individuals collected during the growing season. Five hatchling turtles collected in Illinois in 1939 and 1940 had grown as follows: plastron length 2.20 cm. at hatching and had increased 1.10 cm. to June 30; Pl. 2.54 cm. and had increased 1.53 cm. to July 5; Pl. 2.77 cm. at hatching and had increased 1.44 to Aug. 19; Pl. 2.73 cm. at hatching and had increased 0.67 cm. to June 8; Pl. 2.28 cm. and had increased 1.22 to June 29. Other hatchlings taken during the summer months had not increased in length.

These data suggest that there is no significant difference in the growth increments for the first season between the Louisiana, Illinois and Michigan populations. Thus the northern turtles must have the potentiality of more rapid growth to utilize the short growing season or there may be no real difference in the length of the growing season. The latter possibility appears to be the more probable one.

Growth after the first season is extremely variable. Smaller individuals have a potentiality for more rapid growth than larger individuals. Of 27 individuals marked and recovered after 11-12 months (one growing season) in Nigger Creek, Michigan only 18 increased in plastron length and only the smaller individuals grew rapidly. Two juveniles increased 10.0 and 12.5 mm. respectively but mature individuals grew only 1-3 mm. except for one female which had an increment of 6 mm. Of those females greater than 13 cm. in length, only one grew in excess of one mm. (the smallest that could be measured).

None of 4 in the size range 10-13 cm. released in southern Illinois for 11-13 months grew. A male in the 9-10 cm. group increased 4.5 cm. in the same period; a juvenile in the 4-5 cm. group increased 2.6 mm.; a female in the 8-9 cm. group increased only one mm.

The variation in growth rate is further demonstrated by the correlation of growth rings and plastron length. Growth rings were clearly discernible on 116 individuals collected at the beginning or end of a growing season. A similarity in correlation of size range and mean size with the number of completed seasons between the Michigan, Illinois and Louisiana turtles is evident (table 1).

Attainment of sexual maturity.—A female is considered sexually mature if

TABLE 1.—The correlation of plastron length and the number of completed growing seasons in juveniles

	First Season			Second Season		
	No.	Range cm.	M	No.	Range cm.	M
Michigan	32	2.85-4.76	3.90	13	4.21-5.70	5.01
Illinois	26	3.30-5.75	4.21	11	4.21-7.10	5.40
Louisiana	12	3.17-5.60	3.87	3	4.78-6.17	5.52
	Third Season			Fourth Season		
	No.	Range cm.	M	No.	Range cm.	M
Michigan	7	5.33-6.69	5.92	2	6.17-7.58	6.87
Illinois	2	4.96-8.35	6.66	..	.....	.....
Louisiana	6	5.5-9.0	6.75	2	9.3-9.9	9.6

capable of depositing eggs during the growing season in which she is collected. It is assumed that any female having ovarian follicles with a diameter 1.0 or greater is capable of depositing eggs. Of 127 Illinois females examined by dissection 20 containing eggs were 12.6-16.0 cm. in plastron length; 41 containing follicles greater than 1.0 cm. in diameter were 12.2-16.2 cm. in plastron length; the remainder were juvenile females not exceeding 12.5 cm. in plastron length. Fourteen egg-containing Tennessee females were 10.6 to 13.8 cm. in plastron length. A single Michigan female with oviducal eggs was 15.2 cm. in plastron length. Of 16 Louisiana females collected in April the single one containing follicles greater than 1.0 cm. was 12.5 cm. in plastron length; others with plastron lengths of 9.0-11.3 contained follicles 5 to 8 mm. in diameter; turtles less than 9.3 in plastron length all had follicles less than 5 mm. in diameter. Females become sexually mature when they attain a plastron length of 12.0-13.0 cm.

Logier (1941:105) reports that nesting females from Prince Edward County, Ontario were 14.1-16.5 cm. in carapace length (plastron length 13.13-15.53?); these lengths average 0.97 cm. less than carapace lengths in mature females from Illinois.

The most conspicuous secondary sex characteristics of the males are the elongated nails of the fore feet and the long preanal region. Measurements were made of the length of the 3rd toe on the fore feet and the distance from the rear edge of the plastron to the anal opening. Dissection of males indicated that occasional individuals may be sexually active before the nails or the preanal area is conspicuously longer than those of the female but all males having the elongated nails and preanal area were sexually mature.

The males in the Michigan population become sexually mature when they attain a plastron length of about 9 cm. (fig. 2). The males of the southern

Illinois population become sexually mature at a plastron length of about 7 cm. (fig. 2). One Louisiana male was sexually mature at a plastron length of 5.5 cm., another at 6.23 cm. but other males in the size range 5-6 cm. were not sexually active.

The males of the southern population apparently may become sexually mature during the first year of life (in one complete growing season). The northern males require at least two and possibly three seasons to attain maturity.

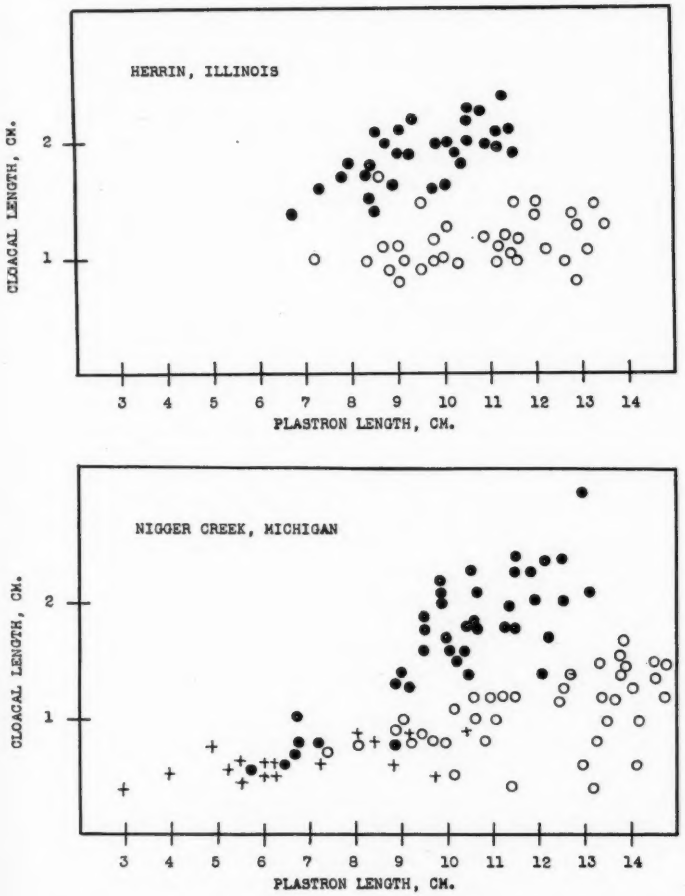


Fig. 2.—The relation of preanal length (cloacal length) to plastron length in Illinois and Michigan turtles. Solid circles indicate males, hollow circles females and cross marks individuals in which sex was not determined.

Annual activity cycle.—Field observations in Illinois during the period 1937-1942 provide no basis for the suggestion of any significant deviations from the annual activity cycle of turtles of the genus *Pseudemys* in Illinois (Cagle, 1950) It is tentatively assumed that the painted turtles become active when water temperature becomes higher than 10° C. and that the optimum temperature falls within the range of 20-25° C. Activity is probably not sufficient to permit measurable growth (1 mm.) in plastron length prior to the end of May in Illinois or prior to the end of February in Louisiana. Similarly, activity is probably not so reduced as to inhibit growth in plastron length until the end of October in Illinois and the end of December in Louisiana. The growing season in Louisiana is thus possibly 5 months longer than that in Illinois. The growing season in northern Michigan is probably restricted to the months of June and July. These assumptions are based upon cycles of water temperatures. It is certainly pertinent that Evans and Hegre (1940) and others have suggested that winter quiescence in reptiles may be independent of temperature and that a genetic time factor may be operative.

The egg laying season is defined here as that period in which females may contain oviducal eggs. Such a period can be delimited by the examination of seasonal samples of females in the size range of sexual maturity. Of 26 egg-containing females collected in southern Illinois the earliest was taken May 12 and the latest July 22. All females within the size range of sexual maturity collected in Illinois and Tennessee during June either had eggs in the oviducts or had recently deposited them. Such females retained ovarian follicles 1.3 cm. or greater in diameter. Of 28 large females from Havana, Illinois, June 10, 1941, 15 contained eggs and the remainder had deposited a brood or had ovocytes ready for ovulation. Of 9 large females collected at Havana, Illinois, June 7, 1943 only two contained oviducal eggs. No such females were found in April or August although many were examined. An egg-containing female was taken in the Huron River in Michigan on May 2, earlier than any were collected in southern Illinois, but the eggs did not have the shell formed and it is not known how long the eggs would have remained in the oviducts. Sixteen of 18 females collected at Reelfoot Lake, Tennessee, June 23-28, 1940 contained oviducal eggs. The only two such females collected in Louisiana were found in June but local egg collectors insist that the painted turtle lays from early April to the last of July.

Observations of actual nesting may be used to supplement the data on egg-containing females. The following dates for nest construction have been reported:

Thacker (1924) British Columbia "First nest observed" May 31, 1922; Breckenridge (1944), Minnesota, 1st week in June, 1938, July 7, 1940; Richmond and Goin (1938) Virginia, May 16, 17.

Cunningham and Huene (1938:382) report the presence of eggs within the oviduct June 7 and June 27 in New York.

Mortality.—The mortality in winter nests in the extreme northern United States and Canada is probably great. However, few reports are available. Breckenridge (1944:182) indicates that one group of eggs laid in Minnesota July 7, 1942 and examined May 9, 1943 had nearly full-grown embryos dead in the shell.

REFERENCES

AGASSIZ, LOUIS 1857—Contributions to the natural history of the United States of America. First Monograph. 51, 643 pp., 34 pls.

BISHOP, SHERMAN C. AND F. J. W. SCHMIDT 1931—The painted turtles of the genus *Chrysemys*. Field Mus. Nat. Hist. Zool. Ser. 18:123-139.

BLANCHARD, FRANK N. 1921—The amphibians and reptiles of Dickinson County, Iowa. Univ. Iowa Studies in Natural History 10:19-26.

BRECKENRIDGE, W. J. 1944—Reptiles and Amphibians of Minnesota. University of Minnesota Press:1-202.

CAGLE, FRED R. 1937—Egg laying habits of the slider turtle (*Pseudemys troostii*), the painted turtle (*Chrysemys picta*), and the musk turtle (*Sternotherus odoratus*). J. of Tenn. Acad. Sci. 12(1):87-95.

———1942—Turtle populations in southern Illinois. Copeia (3):155-162.

———1945—Recovery from serious injury in the painted turtle. Ibid. (1):45.

———1946—The growth of the slider turtle, *Pseudemys scripta elegans*. Amer. Midl. Nat. 36(3):685-729.

———1950—The life history of the slider turtle, *Pseudemys scripta troostii* (Holbrook). Ecol. Monogr. 20:31-54.

——— AND A. H. CHANEY 1950—Turtle populations in Louisiana. Amer. Midl. Nat. 43(2):383-388.

CAHN, ALVIN R. 1937—The turtles of Illinois. Cont. Zool. Lab. of U. of Ill. (492):1-218.

CONANT, ROGER 1938—On the seasonal abundance of reptiles in Lucas County, Ohio. Herpetologica 1(5):137-144.

——— AND ROBERT G. HUDSON 1949—Longevity records for reptiles and amphibians in the Philadelphia Zoological Garden. Ibid. 5(1):1-8.

CUNNINGHAM, BERT AND ELIZABETH HUENE 1938—Further studies on water absorption by reptile eggs. Amer. Nat. 72:380-385.

EVANS, LLEWELLYN T. AND ERLING HEGRE 1940—Endocrine relationships in turtles. Endocrinology 27(1):144-148.

FINNERAN, LEO CHARLES 1948—Reptiles at Branford, Connecticut. Herpetologica 4:121-160.

HARTWEG, NORMAN 1944—Spring emergence of painted turtle Hatchlings. Copeia 1:20-22.

LOGIER, E. B. S. 1932—Some account of the amphibians and reptiles of British Columbia. Trans. Royal Canadian Institute 18:311-336.

———1941—A faunal investigation of Prine Edward County, Ontario. University of Toronto Studies, Biological Series (48):5-106.

LYNN, W. GARDNER AND THEODOR VON BRAND 1945—Studies on the oxygen consumption and water metabolism of turtle embryos. Biological Bulletin 88:112-125.

MARCHAND, LEWIS J. 1942—A contribution to a knowledge of the natural history of certain freshwater turtles. Thesis. University of Florida:1-82.

NEWMAN, H. 1906—The habits of certain tortoises. Journ. Comp. Neurol. and Psych. 16:126-152.

NICHOLS, J. T. 1933—Further notes on painted turtles. Copeia 1:41-42.

PEARSE, A. S. 1923—The growth of the painted turtle. Biol. Bull. 45:145.

RANEY, EDWARD C. AND ERNEST A. LACHNER 1942—Summer food of *Chrysemys picta marginata* in Chautauqua Lake, New York. Copeia 2:83-85.

RICHMOND, NEIL D. AND COLEMAN J. GOIN 1938—Notes on a collection of amphibians and reptiles from New Kent County, Virginia. Annals of the Carnegie Museum 27:301-310.

SMITH, HOBART M. 1950—Handbook of amphibians and reptiles of Kansas. Misc. Publ. No. 2, Univ. Kansas Museum of Nat. Hist. 1-336.

THACKER, T. L. 1924—Notes on Bell's painted turtles (*Chrysemys marginata bellii*) in British Columbia. Can. Field Nat. 38:164-167.

Notes on the Shorttail Shrew (Blarina brevicauda kirtlandi) in Northern Lower Michigan

William O. Pruitt, Jr.
Arctic Aeromedical Laboratory, Ladd Air Force Base, Fairbanks, Alaska

During the course of a study of microclimatic and edaphic factors affecting the local distribution of the shorttail shrew in northern Lower Michigan, quadrat trapping of a series of five study areas resulted in the collection of considerable data on this animal. Three of these study plots were on Rubicon sand, and illustrated the succession from a recently burned area, through the aspen association to the re-establishment of the pine climax. The fourth area was in essentially virgin Northern Hardwoods on Emmet sandy loam, while the fifth was in a mature white cedar bog on Carbondale muck. These five areas are representative of the major small mammal habitats of the region. They are referred to hereafter as *burn, aspens, pines, hardwoods,* and *bog.* These successional stages are analyzed in detail by Gates (1926) and Braun (1950:353-354).

The results of these microclimatic and edaphic investigations are embodied in a paper to be published separately. Very briefly, that study disclosed the fact that, in the region studied, *Blarina* was not distributed uniformly over the region, but was localized in pockets or residual centers, where it was restrained from invasion and ecesis of the neighboring areas because of winter freezing of the soil and excessive heat and dryness in the summer. These population centers are characterized by *year-round* suitability of the environment, i. e., soil temperatures are not above the lethal limit for *Blarina,* soil moisture conditions are such that free water or water of condensation is present, and extensive soil freezing does not occur.

In the region studied these conditions were ideally fulfilled only on loams, where the vegetation was mature deciduous forest, and where the ground cover and litter was deep and friable. Of the five study areas under observation, only one (hardwoods) fulfilled these conditions. Table 1 shows the day-by-day results of the trapping periods on the several areas.

Age

Pearson (1945), in his study of longevity of *Blarina,* reviewed the methods of age determination which have been used in various studies of this animal. He concluded that tooth wear, with certain reservations, was the most reliable criterion of age, and that such criteria as weight, length, condition of reproductive organs, scars, and hairiness of the tail could be used only as corroborative evidence of age. The general unsuitability of weight and length as age indicators was again demonstrated in this study. Analysis of the standard skull measurements as compared with tooth-wear classes of these *Blarina* revealed no significant or definite correlation, other than a general enlargement with age. The exception to this was interorbital breadth, which apparently decreased with age.

The great objection to tooth-wear as an age indicator stems from possible variation in abrasion due to differential food-habits of different individuals. This objection is largely overcome in the present study because the principal

Blarina population occurred in a homogeneous environment where any unusually abrasive food supply available to one individual would have been equally available to all.

Jackson (1928), in his classic study of *Sorex* and *Microsorex*, recognized four age classes: immature, young adult, adult, and old adult, on the basis of a summation of characters including shape of cranium, sutures, ridges, tooth wear, and shape of incisors.

Davis (1941) used three age groups characterized by "considerable wear," "medium amount of wear," and "little or no wear" on P^4 in his study of *Cryptotis* in Texas. He found that he could also separate young individuals from the other two classes on the basis of ratios of palatal length/skull length and maxillary breadth/skull length.

Pearson (*op. cit.*) published photographs of his seven classes of tooth wear. Unfortunately, the photographs were not well reproduced in publication. Through the kindness of Dr. Pearson I was able to obtain the original negatives of these photographs and use enlargements from them as an aid in this study.

The teeth were examined in great detail under a binocular microscope, and records kept of wear on each cusp. Summary of these records revealed the following wear pattern. Class 1 shows no appreciable wear on any teeth, all cusps sharp and well-defined. Class 2 has slight wear on some cusps, with others still unworn. Class 3 has distinct wear on some cusps, except the hypocone-metacone ridge of M^3 and only slight wear on the posterior-medial cingulum. Class 4a has distinct wear on all cusps, and in addition the mesostyles 1 and 2 of M^2 are worn down to base level and are indistinguishable, while mesostyles 1 and 2 of M^1, although showing distinct wear, are still identifiable as entities. Class 4b has distinct wear on all teeth, and mesostyles 1 and 2 of both M^2 and M^1 are worn down to base level and are indistinguishable. Class 5 has all cusps well worn, and some replaced by a connecting groove (especially protocone, protoconule, and metaconule of M^2 and M^1). In addition the parastyle and parastylule of P^4 are indistinguishable. I use the term "parastylule" to designate the small cusplet on the medial slope of the parastyle. Class 6 has all cusps well worn, and some replaced by a groove (especially the lateral cusp group consisting of metastyle, metacone, mesastyles, paracone, and parastyle replaced by one continuous groove and the medial cusp group of protoconule, protocone, and metaconule replaced by another continuous groove). In addition, on P^4 the parastyle, parastylule, and protocone are worn away and replaced by a connecting groove. Class 7 has all teeth well worn. The molar occlusal surfaces are worn essentially to a base level from which weakly project the remnants of metacone, hypocone, and protocone. In class 7 the upper first incisors may be worn down to mere stubs which are apparently incapable of performing their supposed "forceps-like" function.

From several applications of this method I have found that the upper dentition shows less variation in wear than does the lower, and that M^1 shows the least variation of any of the upper teeth, with P^4, M^2, and M^3 following in order of increasing individual variation.

On my hardwoods area I found no *Blarina* in wear classes 1, 6, or 7. This is not remarkable, since at the time of trapping (24-29 June) the shrews sur-

TABLE 1.—Day-by-day catches of *Blarina*. H=Hardwoods, P=Pines, S=Bog, A=Aspens, B=Burn

	AGE GROUPS							
	1	2	3	4a	4b	5	6	7
Jun. 23								
(H-1) 24			1♀	3♂,4♀	3♂,5♀			
(H-2) 25			2♀	1♂,2♀	2♂,2♀	1♀		
(H-3) 26		1♀	1♀	1♂	1♀	1♀		
(H-4) 27					1♂,1♀			
(H-5) 28				3♂				
(H-6) 29				1♀		1♂,1♀		
30								
Jul. (P-1) 1		1♂	1♂,1♀		1♀			
(P-2) 2								
(P-3) 3								
(P-4) 4								
(P-5) 5								
(P-6) 6							1♂	
7								
8								
(S-1) 9								
(S-2) 10								
(S-3) 11			1♀					
(S-4) 12							1♀	
(S-5) 13								
(S-6) 14				1♀		1♂		
(A-1) 15								
(A-2) 16								
(A-3) 17			1♀					
(A-4) 18								
(A-5) 19				1♂				
(A-6) 20				1♂				
21								
22								
(B-1) 23								
(B-2) 24								
(B-3) 25								
(B-4) 26								
(B-5) 27								
(B-6) 28								

viving from the previous year were probably in wear class 5, and the young of the year were already about two months old. It may be seen from table 1 that the great bulk of the population falls into my classes 4a and 4b, which are subdivisions of Pearson's class 4. It is apparent that this frequency distribution closely fits a normal curve and appears to be a stable breeding population. It should be noted, however, that the non-hardwoods *Blarina* do not follow this pattern. They show a tendency to be young or old, with comparatively few in the "middle-aged" classes. This is a further confirmation of the thesis that the main population center is in the hardwoods, with the populations of the other areas composed largely of invaders, either young individuals or old ones.

BREEDING

Hamilton (1929) noted that in New York State male *Blarina* bred when the testes reached 9-10 mm. in length. The average length of testes of non-

breeding males was 2-3 mm. No hardwoods area male approached breeding condition during the trapping period 24-29 June. Average testes length was 2.7 mm., maximum was 5 mm. Aspen area males are also non-breeding (2.25 mm.) on 15-20 July. However, the pine area males (1-6 July) are both in breeding and non-breeding condition (2, 4, and 10 mm.), and the single bog area male (9-14 July) was in full breeding condition. (10 mm.)

Hamilton gives March and April as the spring rutting months for *Blarina*. Manville (1949) gives a slightly later date (May and June) for the Huron Mountains in northern Michigan. A male taken on 22 December near the hardwoods area, in an extension of the same habitat (WOP no. 296) had testes measuring 9 mm. in length, indicative of approach to breeding condition. In Cheboygan County I have taken gravid females only twice; on 1 July 1948 and again on 12 July 1951 (no. T-47, age class 6). T-47 was taken in the bog area, and the 1948 specimen was from the "Gorge," an area with a very cool, moist, shaded and stable environment, which is continuous with the 1951 bog area.

These facts, along with the large percentage of the hardwoods population that was composed of "young adults" during the trapping period of 24-29 June, indicate that in the region studied, *Blarina* does not have a simple, uniform breeding season. Apparently this period was completed before mid-June on the hardwoods area (and, if any bred there, on the aspen area, also), and may possibly commence soon after the first of the year. In the bog (and pines, if any bred there) apparently breeding was still in progress during midsummer. No *Blarina* were found on the burn.

In mammals the onset of breeding apparently is controlled by light. In the above-mentioned paper, by means of microclimatic analysis I have shown that the bog area has a much reduced amount of light reaching the forest floor. The apparently retarded breeding of the bog *Blarina* confirms this analysis.

Another aspect of this phenomenon should be considered. If the general *Blarina* population of the region is subdivided into local pockets or centers which have slightly different breeding seasons, then it follows that gene flow throughout the entire population is not unrestricted. If the population segments are sufficiently segregated, an opportunity is created for differential gene frequency to occur. Possibly this may be an explanation for the rather frequent occurrence of partly albinistic *Blarina* in many parts of its range. As I have shown in the above mentioned earlier paper, this present condition of isolated population pockets has probably existed in such a marked manner only since the days of the lumbermen and subsequent fires (about 80 years). Even so, in the primeval state, some restriction of gene flow between bog and upland populations must have occurred.

SEX RATIOS

Hamilton (*op. cit.*) found that his *Blarina* showed a sex ratio of about 2 males to 1 female. He indicated that this general ratio held for the specimens of *Blarina* in several large museum collections, also. Townsend (1935) noted an apparent differential habitat preference between males and females of *Blarina*. He noted that *Blarina* males appeared to be more numerous in dry woods, while females appeared to be more numerous in moist woods. He called attention to the necessity of noting exact habitats in field notes when

sex ratios are to be computed. He also brought up the question of possible succession in the kinds of small mammals on any area. He suggested that perhaps *Blarina* is a climax species, or that *Blarina* is part of a cycle with *Blarina* "tending to achieve dominance until some disease, parasite, dry season or other cause (or combination of causes) in turn checks it and gives others a chance to start the cycle anew" (p. 74). Manville (1949) noted an approximately even sex ratio in his Huron Mountain study. Unfortunately his sex ratio data were not broken down by habitats. Other workers have also consistently found ratios that were approximately even or in favor of the males.

On all my study areas, the *Blarina* sex ratio was 20 males to 30 females. Moreover, of the hardwood *Blarina*, the ratio was 15 males to 24 females, and in the bog it was 1 male to the 3 females caught. "Three nights" trapping in the hardwood area gave a comparable ratio of 9 males to 22 females. Conversely, on the aspen and pine areas, the ratio was 2 males to 1 female, and 3 males to 2 females, respectively. The very fact that so few *Blarina* were taken on the non-hardwood areas has great biological significance and has been more fully discussed elsewhere. In addition, these few have a sex ratio just the opposite of the hardwoods *Blarina*. It would seem from these data that, in a natural, undisturbed state, *Blarina* has a sex ratio of 1 male to 2+ females. In the disturbed areas of secondary succession, the ratio tends toward reversal, with males the predominant invaders. It must be noted, however, that Dice (1920) trapped only males in Warren Woods, an area of virgin maple, beech, and elm in Berrien County, Michigan.

Study of table 1 reveals some interesting comparisons. Note the changing sex ratio in the hardwoods trapping from a "three nights" catch to the second three nights trapping. The first three nights of trapping caught 21 females and 10 males. Next followed a night with one male and one female, then a night with 3 males, and finally a night with 2 females and 1 male caught. By these data it seems that the normal sex ratio on the hardwoods area is 1 male to 2 females. This stable population was removed in the first three nights trapping, then there was a lull in catches (one male and one female) until the area began to be repopulated, and on the fifth night of trapping only males were caught, by the sixth night the stable sex ratio began to be reestablished. The animals taken on the fourth, fifth, and sixth nights were all caught in peripheral traps, none were taken more than three trap intervals in from the edge of the plot. Thus, after the resident population was removed, there was a marked invasion which was initiated by males and followed by the females.

Such an hypothesis agrees well with the great number of males found by Hamilton (1929) in the various museum collections. Virgin areas are so few and so small that most *Blarina* in study collections probably come from secondary successional stages. This hypothesis also explains the apparent discrepancy between sex ratio and permanent mating. Field evidence points to some sort of more or less permanent mating in *Blarina*, yet this is hard to reconcile with continual presence of more males than females. However, two or three females being served by one male, that has a home range overlapping or adjacent to all of theirs, would be a stable natural condition, and would explain the apparently larger home range of the male, which has been noted by many investigators.

ACKNOWLEDGMENTS

I am indebted to the Regents of the University of Michigan for a University Fellowship during 1951-52 which enabled this entire study to be completed. I am grateful to Dr. W. H. Burt of the Museum of Zoology, University of Michigan, for his aid and valuable criticisms in the preparation of this paper.

SUMMARY

A number of specimens of the shorttail shrew from northern Lower Michigan were collected from study plots which were under microclimatic investigation. These were examined for possible age determination criteria and it was found that tooth wear was the most reliable of any criteria examined.

It was found that the *Blarina* of the region had a complete breeding season, the onset of which varied in different microhabitats. Some of the implications of this complexity are discussed.

The *Blarina* population on an area of virgin northern hardwoods was found to have a sex ratio of 1 male to 2+ females, while the ratio on areas of secondary succession was found to be the reverse. The usual 2 male to 1 female ratio is explained on the basis of males being the primary invaders of areas of secondary succession.

REFERENCES

BRAUN, E. LUCY 1950—Deciduous forests of eastern North America. Philadelphia, Blakiston Co., pp. 1-xiv+596.

DAVIS, WILLIAM B. 1941—The short-tailed shrews (*Cryptotis*) of Texas. Jour. Mamm. 22:411-418.

DICE, LEE R. 1920—The mammals of Warren Woods, Berrien County, Michigan. Occ. Papers Mus. Zool. Univ. Mich. 86:1-20, 1 fig., 2 pls.

GATES, FRANK C. 1926—Plant successions about Douglas Lake, Cheboygan County, Michigan. Bot. Gaz. 82:170-182.

HAMILTON, W. J., JR. 1929—Breeding habits of the short-tailed shrew, *Blarina brevicauda*. Jour. Mamm. 10:125-134.

JACKSON, H. H. T. 1928—A taxonomic review of the American long-tailed shrews (Genera *Sorex* and *Microsorex*). North American Fauna 51:1-238.

MANVILLE, RICHARD H. 1949—A study of small mammal populations in northern Michigan. Misc. Publs. Mus. Zool. Univ. Mich. 73:1-83.

PEARSON, O. P. 1945—Longevity of the short-tailed shrew. Amer. Midl. Nat. 34:531-546.

TOWNSEND, M. T. 1935—Studies on some of the small mammals of central New York. Roosevelt Wild Life Annals 4(1):1-120.

The Young of the Woodland Jumping Mouse, Napaeozapus insignis insignis (Miller)

James N. Layne and W. J. Hamilton, Jr.
Cornell University, Ithaca, N. Y.

The early growth and development of the woodland jumping mouse, *Napaeozapus*, have not been described in adequate detail. Many litters have been born to captive females (Hamilton, 1935) and to females bred in captivity (Sheldon, 1938) but the young are usually eaten by the mother or deserted soon after birth. Sheldon (1938) succeeded in raising one litter to 16 days of age and another to more than ten weeks, but made observations only at rather infrequent intervals.

On June 5, 1952, we took two adult females at Stony Clove (alt. 2000 feet), Hunter Mountain, Greene County, New York. They were kept in large aquaria in the laboratory and gave birth to litters of three and four young each on June 13 and June 20, respectively.

One new born young (CU No. 6849) was removed from the first litter to be described and preserved. The remaining two were examined on June 16. These were subsequently deserted by the female and found dead three days later. They had not been mutilated in any way. In the second litter, one young, noticeably smaller than the others, died on the sixth day; another was killed at 64 days to observe skeletal development.

The adults, and the young after weaning, were fed shelled whole corn, oat flakes, rabbit pellets, strawberries, mulberries, raspberries, lettuce, and apples. The berries were particularly favored. The aquaria were kept covered with several layers of cheesecloth to prevent disturbing the animals with strong light and movements in the laboratory. Shredded toweling was provided for nesting material while the young were small so that they would not easily become separated from the mother. Measurements were obtained up to 26 days of age from photographs in which a centimeter rule was included (Svihla, 1933). Weights of the entire litter were taken at intervals of two to six days until 40 days of age and less frequently thereafter. The weights were divided by the number of young to obtain average weights of individuals. This was done to reduce handling of the young and to permit more time for actual observations during the time they were removed from the nest.

New Born Young.—The umbilical cord is a dried stub about .5 mm. long. The skin is rugose, loose, pinkish in color, and fairly transparent, so that the sutures between frontals and parietals, and the ribs, sternum, and viscera are distinct. Except for vibrissae, the body is hairless, and no dark pigmentation that would indicate aggregation of pigment in hair follicles is seen. In both sexes, the four pairs of mammae are visible as small light spots on the venter. Facial vibrissae are present, although once considered to be absent in zapodids at birth. Quimby (1951) has confirmed their presence in new born *Zapus*. The most posterior mental bristles are about .2 to .3 mm. and grade down in size anteriorly. Those at the edge of the chin are not yet visible. The mystacial vibrissae are arranged in six major rows and show a similar size gradation. Those nearest the eye are the longest, measuring about .3 mm. (fig. 1a).

The sealed pinna of the ear is tightly folded. There is no pigmentation visible and the edge is poorly defined. The eye is visible beneath the skin as a dark ring about 1.5 mm. in diameter containing the pinkish lens vesicle. Blunt, tuberculate claws are present on all digits of the fore and hind feet, with the exception of the thumbs. New born *Zapus* apparently lack claws (Quimby, 1951).

This new born young had the following measurements: weight .87 grams, total length 35.2 mm., tail 11.0 mm., hind foot 5.0 mm., and head length 10.0 mm. Hamilton (1935) gives the average weight of new born *Napaeozapus* as 1 gram, total length 42 mm., tail 12 mm., and hind foot 5 mm., and a specimen obtained by Sheldon (1934) when only a few hours old weighed 1 gm. and had a total length of 39 mm., tail 9.5 mm., and hind foot 5.0 mm.

Soon after birth the young are active when disturbed, particularly when placed upon a cold surface. Movements consist mostly of rhythmical writhing motion of the hindquarters in which the hind feet are held together and moved in unison.

One week.—In the first week of development of the three surviving young there is little change in appearance. At six days the posterior mystacial and mental bristles are about 2 mm. long and the folded pinna is more prominent, with its edge distinctly elevated and dusky. The young utter faint sucking sounds continuously and give sharp squeaks when disturbed.

Second week.—At ten days minute pigment spots are apparent beneath the skin surface on the dorsum of the head, body, and tail. Pigmentation is absent or invisible on the venter of the body and on the tip and underside of the tail; the ultimate pattern of the latter is now evident. The skull sutures are still visible as are blood vessels on the lower sides, belly, and limbs, but the skin has become more opaque and flesh colored. The vibrissae are prominent, the longest mystacial measuring 3-4 mm. The pinna of the ear has unfolded and stands out from the head. It is darkly pigmented on the posterior and upper margins but not below the middle on the anterior edge. The external auditory meatus is still closed. The developing eyes bulge slightly from the surface of the head while the skin covering them is looser and a faint crease which marks the eventual line of separation of the lids is apparent. The claws are less tuberculate and sharper, resembling the flattened claws present on the thumbs of the adult. The young cling tenaciously to the nipples of the mother and can be removed only by carefully manipulating them until they relax their hold. The female does not leave the nest if movements by the handler are slow.

On the 12th day the dorsal aspect of body and tail have become noticeably gray. The tip of the tail is flesh colored. Fine hairs are just visible on the top of the head, the upper part of the body, and sparsely on the limbs. None are visible on the belly and lower sides. The posterior mystacial bristles are approximately 6 mm. long and reach beyond the rear edge of the eye. The black pigmented plantar tubercles of the hind feet contrast with the light areas of the sole. The young exhibit some bodily control. They stand on all feet, though shakily, and can take a few steps before falling over on their sides. A soft, thick covering of fur on the back and sides is present on the 14th day. The hairs are over .5 mm. and are thickest and longest on the

muzzle. There are a few white hairs on the belly, and the skull sutures and viscera are no longer visible through the skin (fig. 1b).

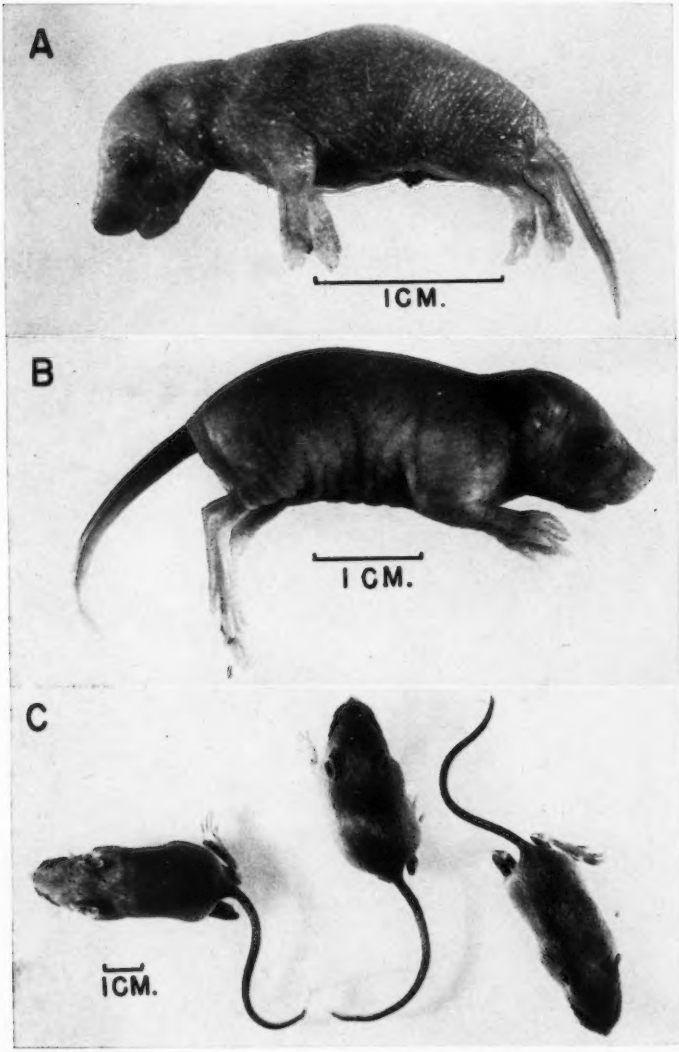


Fig. 1.—A. New born *Napaeozapus*. B. Fourteen day old young. C. Young at 26 days

Third week.—By the 17th day the back is well furred with short, sulfur colored hair and the broad middorsal band has become less distinct. The belly is clothed with sparse white fur. The most posterior series of mystacial bristles extend nearly to the base of the ears.

Two days later the dorsal pelage has grown longer and more luxuriant and now possesses a beautiful golden sheen. The dark middorsal patch is not apparent. The furring of the feet and venter is more sparse than on the back, the pink color of the skin still showing through in these areas. The claws are well formed. The lower incisors are first seen protruding above the gums about .2 to .3 mm.

The proportions of the young definitely show the pattern of a jumping mouse. They stretch, yawn, and sit up unsteadily to "wash." They also attempt leaps of an inch or so but usually land on their sides, futilely kicking. When three weeks old, the belly fur has grown thicker and longer. The middorsal band is still faint but again begins to become more distinct. Furring on the feet is more pronounced and the longest mystacial bristle extends nearly to the forearm. The lower incisors are over .5 mm. long; the upper incisors are just piercing the gum.

Fourth week.—At 24 days the young are well furred dorsally and ventrally although the rudimentary mammae are still visible through the hair. The middorsal band is becoming more conspicuous and the line of demarcation with the yellowish sides is pronounced. The thin and transparent lower incisors are about 1 mm. long and the upper incisors half that length.

The young have now increased in size to the point where the female often nurses them while lying on her side, whereas previously she was invariably found hunched over the young. This is a common characteristic of many small rodents.

The middorsal band is again well defined on the 26th day and the body fur longer throughout, but the teats are still visible on the venter. The eyes open at this age and the external auditory meatus is now definitely unsealed. The young are particularly active and no longer give the soft sucking note. They react to a loud or sharp sound, and one winced when a light was turned on above it. They progress with slow hops, much like a foraging cottontail, with the ungainly hind feet splaying out at about 45 degrees from the body axis (fig. 1c).

At four weeks the lower incisors are about 2 mm. and the uppers about 1 mm. Both are becoming more opaque and yellowish at the base. In addition to the slow hops the young are capable of leaps exceeding a foot. While they were confined to a box for weighing one was heard drumming with the tail, a behaviorism reported for *Napaeozapus* by Sheldon (1938) and for *Zapus* by Svihla and Svihla (1933).

Fifth week.—At 31 days the middorsal band is as definite as in the adult. The teats are no longer visible through the ventral fur. The incisors are yellowish in color and the grooves on the anterior surface of the uppers are prominent for the first time.

When 34 days old the young resemble the adults in action and general appearance, but differ markedly in the color of the sides of the body. In these areas, the adult is much brighter than the young, being almost orange. In contrast, the sides of the juveniles are yellow-brown in color and resemble

Zapus in this respect. Weaning takes place during this period as the young are now observed eating solid food and tend to occupy a different portion of the nest box than the adult.

Little further change is apparent in the young during the following weeks other than general increase in size and weight. When the young and the parent were examined July 28, the former, then 38 days old, frequently attempted to copulate with one another and with the adult. This sexual activity was premature since a 64 day old male had testes measuring only 5.0 x 3.0 mm. and showed no evidence of sexual maturity. Between 63 and 80 days they apparently undergo a molt into the adult pelage since at the latter age the young are indistinguishable in pelage color from the adult.

TABLE 1.—Average measurements from 6 to 64 days of a litter of three *Napaeozapus i. insignis* born in captivity June 20, 1952

Days of Age	Total Length (mm.)	Tail (mm.)	Hind Foot (mm.)
6	48.0	16.0	6.5
10	66.0	18.0	8.0
14	73.0	25.0	12.0
17	89.0	34.0	15.0
19	103.5	40.0	18.0
21	112.0	46.0	19.5
24	123.5	58.0	21.0
26	123.0	70.0	23.0
64	199.0	120.0	28.0

Growth Rate.—Average measurements of the young up to 26 days of age are presented in table 1. Beyond this age measurements could not be obtained with sufficient accuracy from photographs, and it was not considered advisable to attempt anesthesia to obtain measurements from the living animal. Average weights were taken at intervals of two to 41 days and are given in table 2. The greatest relative gain in weight occurred between 10 and 26 days, when the average weight of the young increased from 2.4 grams to 8.5 grams. A slight drop in the rate of gain that occurred between 27 and 31 days is coincident with the period at which the eyes opened and the young began taking solid food.

The growth and development of these young compare generally with data provided by Sheldon (1938) on two litters. She gives the following measurements for a 16 day old specimen: total length 70 mm., tail 31 mm., hind foot 13 mm., while the average of the three young of the present litter at 17 days of age were: total length 89 mm., tail 34 mm., and hind foot 15 mm. A noticeable discrepancy exists in regard to weight. Sheldon (1938) gives 27 and 25 grams as the weight of 47 day old mice in early September. Those of the present litter averaged only 13.5 grams at 55 days. The difference in weights evidently reflects preparation for hibernation. Sheldon's juveniles went into hibernation at about the same time as the adults, whereas in the present case the young did not become definitely torpid until November 3 when they weighed 23.5 and 22.1 grams. The adult in the same aquarium outdoors was in sound hibernation on October 16, and intermittently for several weeks pre-

TABLE 2.—Average weights of young *Napaeozapus insignis*

Age	Weight (Grams)	Age	Weight (Grams)
Birth	.87	28 days	8.8
6 days	1.6	31 days	9.3
10 days	2.4	34 days	9.7
14 days	3.9	40 days	10.6
17 days	5.5	55 days	13.5
19 days	6.0	62 days	14.1
21 days	6.7	81 days	17.4
24 days	7.9	95 days	17.7
26 days	8.5	136 days	22.8

viously. It weighed 22.8 grams on November 3. Both juveniles and adult were active during warm weather until November 30.

The growth of *Napaeozapus* as revealed by the present litter parallels that of *Zapus* as described by Quimby (1951) but may be somewhat slower. Apparently specific points of difference in development are that in *Zapus* unfolding of the pinna occurs during the first week, incisors erupt at 13 days, the eyes open at 22-25 days, and weaning begins. In *Napaeozapus* the pinna did not unfold until about the tenth day; the incisors erupted at 19 days; the eyes opened at 26 days; and the young began to take solid food regularly at approximately 34 days. Growth in weight of these two genera is essentially similar and considerably slower than in various cricetid mice which have been studied.

REFERENCES

HAMILTON, W. J., JR. 1935—Habits of jumping mice. Amer. Midl. Nat. 16(2):187-200.

QUIMBY, DON C. 1951—The life history and ecology of the jumping mouse, *Zapus hudsonius*. Ecol. Monog. 21:61-95.

SHELDON, CAROLYN 1934—Studies on the life histories of *Zapus* and *Napaeozapus* in Nova Scotia. Jour. Mamm. 15(4):290-300.

———1938—Vermont jumping mice of the genus *Napaeozapus*. Ibid. 19(4):444-453.

SVIHLA, ARTHUR 1933—An accurate method for measuring new-born mice. Science 77(1996):331.

——— AND RUTH SVIHLA 1933—Notes on the jumping mouse, *Zapus trinotatus trinotatus* Rhoads. Jour. Mamm. 14(2):131-134.

Hibernation in the American Black Bear*

Arthur Svihla and Howard S. Bowman
University of Washington, Seattle

During recent years considerable interest has been shown in the study of hibernation. However, there seems to be some confusion as to the meaning of the word when applied to different animals. Funk and Wagnalls's dictionary (1949) defines hibernation as the ability to "pass the winter . . . in a secluded place and in a torpid state, as certain animals." Torpid is defined as "having lost, partially or wholly, sensibility or power of motion, as a hibernating animal." Thus the term hibernation may include the *partial* loss as well as the *whole* loss of sensibility or power of motion. Dormant is described as "being in a state resembling sleep; torpid; hibernating in a state of lethargy as bears and some other animals in winter." Lethargy is defined by the same authority as a state of "complete repose or torpor as an animal hibernating."

Although bears are generally considered to be the classical animal which demonstrate hibernation, some authors in recent years (Matson, 1946; Lyman and Chatfield, 1950) have questioned the fact that these carnivores actually pass the winter in a state of hibernation. However, their interpretation of the word is somewhat different from that designated by Funk and Wagnall. Lyman and Chatfield state that for the purpose of their article, "hibernation is defined as a condition characterized by a marked lowering of the body temperature with a concurrent drop in metabolism, heart rate, respiration and other vital functions. By this definition the most famous 'hibernating' animal, the black bear, does not hibernate at all. It is true that the bear spends a great deal of the winter in a dormant condition, but its body temperature always remains high. Although no one has actually taken a bear's temperature to prove this point (for understandable reasons), there is plenty of indirect evidence. For example, many hunters have reported having found bears curled up on the ground with new fallen snow melted from their backs. Moreover, when disturbed, the bears make off, which would be impossible if the body temperature were near freezing. The most decisive proof is that the she-bear gives birth to her cub in midwinter. How she could give birth and suckle the cub with her body temperature allegedly near freezing has never been explained."

Matson (1946) states that "it is a scientifically accepted fact that the carnivorous animals (including bears, 'coons, skunks, badgers, etc.), do not hibernate. They do go into deep sleep, but their respiration, rate of heart beat and body temperature are not drastically reduced as they are in hibernation. They are relatively easily aroused from sleep and seem to have full sensibilities and use of their faculties. Although efforts have been made, I do not know of a successful attempt to take the temperature and pulse of a wild dormant bear. The fact that falling snow vanishes from the back of a dormant bear at low temperatures indicates their body temperature does not fall to a great extent." He describes a bear discovered in its hibernating den

* Acknowledgement is made to the Arctic Aeromedical Laboratory, Ladd Air Force Base, under Air Force Contract AF33(038)-18509 for aid and assistance in making this study.

as follows: "One of the men tossed a pebble at the bear which moved slightly but did not waken. The bear was then poked with a stick. He rose, placed his front feet on the rim of the pit, yawned and opened its eyes blinking in the bright light." This seems to contradict his previous statement that bears are relatively easily aroused and seem to have full use of their faculties. He goes on to say, "the animal heat of large bears which have been hog-dressed and left lying in the snow, is dissipated very slowly. If the oxidation of fat depends upon the intake of air, the slight loss of weight during dormancy would be explained by the slow rate of respiration. This rate has been noted by reliable observers as low as five respirations in two minutes. The average reported seems to be four or five per minute." This statement also seems to contradict the previous one that respiration of bears during hibernation is not drastically reduced.

Surprisingly there are very few published accounts of actual observations on hibernating bears. Smith (1946) gives a good description of the construction of a hibernating den which was located under "drooped and snow-covered branches of a black spruce . . . the lower branches of which drooped from about three feet and almost swept the ground. Under these spreading branches next to the trunk, she scooped out . . . a small depression in the ground and lined it with *Kalmia* and heather leaf. For a roof she bent down the lower branches of the tree and held them there by weaving through the branches some of the material she used in building the den. The ends of the limbs she buried in the ground, piling on top several broken lengths of decayed grey birch tree. . . . The grey birch she must have carried a considerable distance as none grew in the immediate vicinity. The complete job was such that each storm would drift and bank against the back and sides of the den, thus giving added protection against cold north winds. On the southwest the den was left open to serve as a ventilator and to permit the rays of the sun to shine in and help keep her warm. In this den a female and three young the size of grey squirrels were found on January 27." From the description of the young, they were but recently born. As is quite characteristic of bears, the female abandoned the den and young when she was disturbed.

Morse (1937) records some excellent observations of a bear and her yearling cub which were hibernating together in a den at the base of a 3-inch black spruce and a 3-inch balsam fir. The bed was a depression in the sphagnum moss and Labrador tea with no cover other than that of the lower branches of the black spruce. On January 4th, "a light snow which had fallen the night before covered the fur of the animals." The temperature on the previous day he recorded as +22°F. On January 17 he again visited the bears recording the temperature as +15°F at 8:00 am. At 9:17 am he made his observations stating that "an 8 inch snow had fallen January 12 and 13 . . . there was no snow on their bodies. Whether or not the recent heavy snowfall had melted upon contact with the bear's fur coat or had been dislodgd by movements could not be determined." A later visit was made on February 10th at 3:00 pm. The temperature that morning at 8:00 am registered at —4°F. "Seven inches of snow had fallen in the last 48 hours and a small amount of snow lay on the bear's bodies." These field observations indicate that snow will remain on the fur of bears during the winter, contrary to the reports cited by Matson as well as by Lyman and Chatfield.

Through the courtesy and cooperation of the Arctic Aeromedical Labora-

tory at Ladd Air Force Base, Fairbanks, Alaska, a black bear was made available for study. This bear, a yearling cub, had been in captivity since August 1951. It was kept in a large specially constructed pen. On November 15, 1951, it weighed 84.5 pounds. A week or ten days later it was reported in hibernation since food placed in the cage had been untouched during the preceding week. No more food was given it and the bear remained undisturbed until our arrival in early February. At that time we found the bear in a state of dormancy, curled up in a depression it had made in the hay which formed the nest material. Its position was somewhat similar to that of a hibernating ground squirrel, for the body was so curled up that the head was between its forequarters with only the back exposed above the nest material. It was more sensitive to noises such as loud talking and grating sounds than hibernating ground squirrels for at these sounds it raised its head very slowly and looked at us with what appeared to be unseeing eyes. When we remained quiet however it replaced its head between its forelegs and went back into its original state.

By means of a pletysmograph placed between the body of the dormant bear and the sides of the nest depression a respiration rate of 2 to 3 times a minute was recorded. This is much lower than the rate of 10 to 12 times a minute in complete surgical sedation under the influence of Nembutal (Svihla and Horita, 1952). The rate of respiration in this dormant bear is very similar to that mentioned by Matson (l.c.).

Various temperature records were made by means of thermocouples connected to a Brown recording potentiometer. The thermocouples were so placed as to record temperatures: 1) in different parts of the nest box; 2) the nest material; 3) the area immediately underneath the bear); 4) the surface of the bear's fur; and 5) deep within the fur in contact with the bear's skin.

The temperature of the nest material depended upon the nearness of the thermocouple to the body of the bear. If it was placed some distance away, the recording did not differ from that of the ambient air. However, if it was placed near the bear's body the temperature registered higher but not as high as that immediately beneath the bear. During one series of records over a period of 6 hours, the temperature immediately beneath the bear averaged $+40°$F while during another series over a period of 20 hours, it averaged $+50°$F. At the same time the ambient air temperature of the den was approximately $+18°$F.

This relatively high temperature beneath the bear may be attributed to two sources: 1) heat radiated directly from the bear's body, and 2) heat dissipated during respiration, for since the bear's head was thrust down between its forelegs, the nest material would be warmed by the residual heat in the exhalant air. This high temperature under the bear's body would be sufficient to prevent the freezing of new-born young even when the surrounding temperature was very low.

Sometimes the bear assumed other positions than that described above. Once it lay on its side with its head still between its forelegs but with the mouth and lips exposed. By carefully manipulating the rather stiff thermocouple wires, it was possible to place one between the bear's lips. Several oral temperatures of $+95°$F were obtained by this method. Although the body temperature was high as indicated by these oral readings, surprisingly enough

the skin temperature was only +40°F. This wide difference may be due in part to the thick layer of subcutaneous fat. Although adipose tissue itself is no better insulator than other tissue, nevertheless when deposited in and about the peripheral blood vessels, it shields the blood from low external temperatures thereby reducing the loss of radiant body heat.

Another conserver of body heat is the long dense fur coat developed by these animals upon the approach of winter. The insulating value of this fur is well illustrated by the fact that although the skin temperature at the base of the hair was +40°F, yet the temperature at the surface of the hair was +32°F. Furthermore, when snow was sprinkled on the fur, it did *not* melt after a period of 36 hours. This is contrary to the report of Matson as well as that of Lyman and Chatfield as cited above, but confirms Morse's note that snow may remain on the fur of hibernating bears without melting.

Even in active bears the winter coat is a very effective insulator for Rausch (1951) records the killing of a Richardson's grizzly bear at Anaktuvuck Pass, Alaska, the fur of which was filled with ice crystals. He stated that the eskimos formerly believed that bears visited open springs and soaked their fur with water before going into hibernation so that the ice which formed in the fur would keep them warm.

TABLE 1.—Temperatures in degrees Fahrenheit

Air	+18°	Oral	+95°
Nest material	+18°-20°	Rectal under influence of Nembutal	+71°
Underneath bear	+40°-50°	Rectal during dormancy	+95°-96°
Skin	+40°		
Hair surface	+32°		

In order to obtain rectal temperatures, it was necessary to anaesthetize the bear. Since no "squeeze" cage was available, the bear was caught in a net and held while Nembutal at the rate of 30 mg per kilo body weight was injected interperitoneally. Although this excited the bear considerably, the Nembutal took effect within 15 minutes when surgical sedation was attained. At this time the respiration rate was 10 to 12 times per minute. An attempt was made to find the saphenous veins in order to obtain blood samples for blood volume determinations. However the subcutaneous fat layer was so thick even after three months of hibernation that the veins could not be located. A thermocouple was placed in the rectum and held in position by adhesive tape wrapped around the base of the tail. The bear was then replaced in its bed and temperature records were made continuously for 28 hours. Under the influence of Nembutal, the body temperature dropped to +71°F. The effects of the anaesthesia wore off within 2 hours and the body temperature rose very gradually to a maximum of +96°F and then stayed rather uniformly at approximately +95°F for another 14 hours. During all this time the bear remained quiet in its nest in the position in which it was first placed. It apparently passed directly from the state of sedation into that of dormancy. This is substantiated by the fact that these rectal temperatures correspond with the oral temperatures of the bear when dormant (table 1). Our records are somewhat higher than those of Hock (1952) who recorded a temperature of only +85°F for a hibernating bear. The high body temperature of bears while

hibernating is in direct contrast to that of hibernating ground squirrels for the latter have body temperatures usually only a few degrees above freezing.

That the general metabolic activities of the black bear are greatly reduced during dormancy in spite of the high body temperature maintained is demonstrated in two ways. First, by the cessation of digestive and kidney activity, for there was no evidence that either urinary excretion or defecation took place during the period of dormancy, and second, by the relatively slow rate of weight loss during the same period. The bear weighed 84.5 pounds when it went into hibernation in November, yet 3 months later, (March), during which time no food was taken, it was still fat and weighed 62.75 pounds. It had lost only about 25% of its weight during these months.

Hunters' accounts vary as to the physical condition of bears in spring when they emerge from hibernation. Some state that the animals are emaciated at this time while others report them to be fat. These seemingly contrary statements may both be correct since some bears may go into hibernation in the fall with less stored fat than others.

In conclusion it may be stated that bears do hibernate for they pass the winter in a dormant state, even though their sensibilities and powers of motion are only partially lost. Their metabolic activities are reduced. Their body temperatures are high but their respiration rates are low. They differ markedly from dormant ground squirrels which have low body temperatures and lose their sensibilities and powers of motion to a much greater degree. Since there is variance in the interpretation of the term hibernation, it seems necessary to designate two types when referring to animals, one which may be called "partial dormancy or torpor" and another which may be called "complete dormancy or torpor." As examples, the bear demonstrates the former while the ground squirrel, hamster and hedgehog, among others, exhibit the latter.

REFERENCES

HOCK, RAY J. 1951—Rectal temperatures of black bear during its hibernation. Second Alaska Science Conference, 1951.

LYMAN, C. P. AND P. O. CHATFIELD 1950—Hibernation. Scientific American 183:18-21.

MATSON, J. R. 1946—Notes on dormancy in the black bear. Journal of Mammalogy 27:203-212.

MORSE, MARIUS A. 1937—Hibernation and breeding of the black bear. Ibid. 18:460-466.

RAUSCH, ROBERT 1951—Notes on the Nunamiut Eskimo and mammals of the Anaktuvack Pass region, Brooks Range, Alaska. Arctic 4:147-195.

SMITH, BERTRAND E. 1946—Bear fatcs. Journal of Mammalogy 27:31-37.

SVIHLA, ARTHUR AND AKIRA HORITA 1952—Use of Nembutal as an anaesthetic for large wild mammals. Science 115:503.

Notes and Discussion

Techniques for Rearing Culex Mosquitoes

The value of mosquitoes as laboratory animals is becoming increasingly widely known. Many laboratories have maintained colonies of *Anopheles* mosquitoes in connection with various problems of malarial research, and some colonies of *Aedes* have been maintained for similar reasons. *Culex* mosquitoes have been reared in various laboratories but the techniques for rearing this genus are not as well known as the standard techniques for *Anopheles*. With the increased realization of the utility of the genus *Culex* in experiments in genetics, evolution and speciation, as well as in classical parasitological work, it seems important to consolidate some of the information pertaining to *Culex* rearing. Much of this information is scattered in "material and methods" sections of various papers, and much of the rest is not available in print. Accordingly, it is somewhat difficult for individuals who wish to use *Culex* mosquitoes as a laboratory stock to discover the short cuts and techniques that are standard practice in the laboratories maintaining *Culex* colonies. Often the techniques for propagation of this species overlap those used for *Anopheles*, but many of the techniques are not interchangeable.

The authors do not claim that any or all of the methods described below are original. In cases in which methods have been described citations are given; for most of the rest of the methods they are the ones in successful use in our laboratories. It should be noted that the limited data available on the differences between strains indicate clearly that some inbred stocks of *Culex* mosquitoes survive much better under laboratory conditions than do others. Strict culture *by the book* therefore does not necessarily guarantee a successful colony.

Following are some methods which have been found successful. They are grouped by subject matter insofar as possible.

Choice of room.—Ideally a basement room would seem best for the maintenance of a *Culex* colony inasmuch as temperature and humidity are easier to control than in most other rooms. From a practical standpoint, however, any available room can be adapted to the rearing of mosquitoes. Although no precise data have been kept, casual observations upon colonies in basement rooms indicate that summer temperatures in some localities are often too cool (without artificial heat) to permit breeding, and that molds and fungi, very deleterious to larvae, develop readily.

When it is desirable to maintain two or more mosquito colonies, the rearing room or rooms should have one or more screened partitions dividing the area into separate units. Although interconnecting, screened doors between the sub-rooms are generally satisfactory, contamination of the individual colonies is largely eliminated if space permits a separate entrance into each. These rooms should contain shelves for rearing cages and pans. Theoretically, mosquitoes are not found free in the rooms. The partitions were originally designed to restrict any escaped mosquitoes to one area and thereby decrease the chance of contamination of other stocks. In practice however, very few adults escape the rearing cages and the chief value of the screened partitions we feel, is to prevent contamination of the stocks with local mosquitoes. There is no question however, that the separate rooms are useful in rearing species which are more eurygamic.

Temperature requirements.—There seems to be little doubt that *Culex* stocks vary considerably in temperature requirements. The *Culex pipiens* and *Culex quinquefasciatus* colonies in our insectaries are maintained at approximately 29°C which seems to be the optimum temperature for development. Temperatures below 27°C slow down the life cycle considerably and mating is not as successful. Colonies of both species have been maintained at temperatures as low as 16-18°C but the length of the life cycle is considerably greater and the mosquitoes more difficult to maintain. Temperatures above 32°C for more than 3 or 4 days are fatal to about 90% of the *Culex pipiens* and interfere somewhat with the development and life span of *C. quinquefasciatus*, although the latter mosquitoes are somewhat more heat resistant. Thermostatic control of heat from a central heating plant is desirable during the winter but not absolutely necessary. With such an arrangement, the temperature variation does not usually exceed 2°C. A window fan may be used to help maintain circulation of air throughout the room.

Humidity.—It is a well known fact that *Culex* as well as other mosquitoes do not thrive under conditions of low humidity. In most sections of the country, it is necessary to raise the humidity of the mosquito rearing room in order to prevent undue mortality. This is especially true during the summer when an insectary must utilize air conditioning

to lower room temperature, since in so doing the air is greatly dehumidified. Several layers of paper towels may be placed on top of the cages and usually need to be saturated with water only once or twice a day to maintain adequate humidity. Cloth towels may be tacked on the sides of the cages and wet down daily to provide still higher humidity. One simple and cheap device which works very well in winter is a large flat pan set on top of the radiator. This is filled twice a day and maintains a sufficient degree of humidity to permit the colonies to live and reproduce. Both species of *Culex* have been successfully reared at 100 per cent relative humidity; however, 75 to 85 per cent seems to be optimum.

Light.—One of the chief differences between the autogenous and anautogenous strains of *Culex pipiens,* as defined by Roubaud (1933, Ann. Sci. Nat. Zool. 16:5-168) is the presence of an obligatory period of hibernation in the anautogenous variety. Roubaud considers that the normal course of events in *Culex pipiens* is several generations of normal reproduction followed by an obligatory ovarian diapause during which the females hibernate and cannot resume normal reproduction until several months have elapsed (heterodynamy). These conclusions have been substantiated by several investigators and one of the chief difficulties in maintaining colonies of *Culex pipiens,* (as distinguished from *molestus-autogenicus*) has been the tendency of the adults to cease reproductive activity during the winter. Such colonies usually die, and it has often been the case that *Culex pipiens,* collected in the spring or early summer, can be maintained for four to six generations in the laboratory but then are lost when they go into a period of hibernation.

Our experience with *Culex pipiens* has been similar to that reported by Tate and Vincent (1936, Parasitology 28:115-145). We have maintained anautogenous colonies of this species for several years in the laboratory, but it is necessary to supply artificial illumination during the winter months. One strain of *pipiens* is presently in the F_{26} generation of strict brother x sister mating, and is entirely normal and active. The ceiling light in the insectary (150W) is left burning from November 1 to April 1 (Urbana) and in addition, a 100W desk lamp is placed near the caged *pipiens.* These lights are left burning night and day except for the nights on which pigeons are placed in the cages. Under this regime the females engorge and lay egg rafts without difficulty. Tate and Vincent (*op. cit.*) have carried out controlled experiments which show definitely that continuous light during the winter months favorably affects gorging and oviposition in their English strain of anautogenous *Culex pipiens.*

The data on our strains of *pipiens,* however, indicate that the genetic basis of the tendency to hibernate during the winter exists, regardless of the fact that its expression is capable of modification by means of light. During the fall of 1949 a strain maintained in a room under conditions of normal daylight and darkness began to show greatly decreased fertility. Egg rafts were fewer, the percentage of fertile rafts decreased, and mating and gorging took place only rarely. At this point the lights were left on day and night, and almost immediately the strain returned toward "normal" reproductive activity. It seems clear therefore, that the light is modifying the expression of the ovarian diapause which seems to be characteristic of this species. We have experienced no difficulty in maintaining *Culex* colonies either in Baltimore or Texas during the winter months. No marked variation in mating or egg production was noticed from one season of the year to the next. Although mosquitoes feed best at night, it has been possible on many occasions to place them in a completely darkened room during the day and induce large numbers of them to take a blood meal. Some strains will feed freely even in full daylight.

Cages.—Stock colonies in our laboratories are kept in cages 12 inches and 24 inches square, covered with no. 18 or no. 20 Bronze screen wire. Usually cages are constructed with a wood bottom and frame, although the smaller type may be built with a heavy wire frame mounted on a wood bottom and covered with screen wire or bobbinet. A cloth sleeve is attached to an opening at the bottom half of the front of the cage to permit pupae to be placed within, or to allow the removal of eggs or adults without freeing the latter into the room. Our strains of *Culex pipiens* and *C. quinquefasciatus* survive and breed very well within these small cages, in sharp contrast to many reported statements that large areas are necessary for mating.

Repeated attempts to breed *Culex restuans, C. tarsalis* and *C. salinarius* in both small cages and room-sized cages have failed. Egg rafts and larvae of these species were collected and the adults could be maintained in cages for a number of days. Although large numbers of them could be induced to take a blood meal, mating and egg laying did not occur.

Care of adults.—Although it is possible to rear *Culex pipiens* and *Culex quinquefasci-*

atus with little apparent contamination in a partitioned room, it has been more satisfactory to keep them in separate rooms which are quite some distance apart or separated by several doors. In order to maintain a stock colony, egg rafts are chosen at random and the adults raised therefrom are used to replenish the colony. This method is thought to be the best approximation to natural conditions. Special methods are used to derive homozygous stocks.

Newly hatched adults of the same generation are liberated in the cages. A pan of water (4 x 15 cm) and a moistened prune are placed in each cage. Prunes have been found to be the most satisfactory food for the adults. One prune is placed in a two-inch stender dish and covered with water. As it absorbs moisture it becomes turgid and is taken readily by both males and females. Raisins and apple slices have also been used as food for the adults, but are no better than prunes and more difficult to handle. Cotton soaked in sugar solution is also satisfactory. Both males and females may be sustained for several weeks on such a diet.

After a minimum of four days to permit fertilization, a source of blood is placed in the cage and left overnight. Although both species will feed on mammalian blood, birds such as a chicken, duck, or pigeon are much more satisfactory. Woke (1937, J. Parasit. 23:311-313) allowed a number of *C. pipiens* to feed upon a canary and a comparable number to ingest human blood. He found that over twice as many eggs per mass or milligram of blood ingested were produced by mosquitoes fed on canary blood. In order to obtain a good feeding, the prune or sugar solution is removed from the cage at least 24 hours before the bird is placed in the cage.

It is important to render the bird relatively immobile so that it will not frighten the mosquitoes away or kill them. This is accomplished by taping the bird in a cylindrical piece of hardwood cloth or upside down in an instrument pan (9 x 22 x 4 cm) after removing feathers from a sizeable area in the pectoral region. The immobilized bird, breast up, is placed in the cage and allowed to remain overnight, although some strains will take blood immediately, even during daylight. The following morning the bird is returned to its cage and the number of engorged mosquitoes noted. Pigeons, which are numerous in almost every urban area and are easy to trap and maintain, provide an excellent source of blood. A single bird may be used once or twice a week for more than a year if so desired.

If the colony is a very large one and the bird fairly small, it must be watched more closely since death may result from exsanguination. If the "blooded" mosquitoes are removed to a separate cage, allowed to lay their eggs and are offered a second blood meal, it is difficult to obtain more than a 35 per cent feeding. Relatively few will take a third blood meal. If mosquitoes are not allowed to complete a blood meal, the egg rafts produced are small (75-125 eggs) as compared to those from females which ingest all the blood they want, averaging 225-250 eggs per raft. All stocks in our laboratories are definitely anautogenous, inasmuch as repeated attempts to obtain egg rafts in both species without a blood meal have failed. It appears therefore that our stocks of *Culex pipiens* are *pipiens* (*sensu strictu*) and not *molestus*. For a general review and discussion of this problem the reader is referred to Mattingly, *et al.* (1951, Trans. R. Ent. Soc. Lond. 102(7):331-382).

Egg rafts.—Egg rafts are deposited about five days after the blood meal. The females usually select the surface of the water in the pan for oviposition, although egg rafts are often found in the prune juice. Each cage is examined daily for rafts and when present, each raft is removed for hatching to a two-inch stender dish or other container filled with water. A few grains of dry powdered yeast may be added to each dish. Preliminary results seem to indicate that more rafts hatch, and more quickly, with the addition of the yeast. The rafts hatch 24 to 48 hours after deposition, and upon hatching the larvae are removed to rearing pans.

Larvae.—Although the larvae are usually reared in tap water, it is necessary in some localities to use distilled water because of the high content of chlorides and other compounds which exert a deleterious effect upon the larvae. Two rafts are placed to a 23cm x 31cm x 4cm white enameled pan containing 2 cm of water. More than two rafts in this size pan results in overcrowding. The larvae are fed with a sprinkle of yeast or of finely powdered dog biscuit immediately upon hatching and each pan is marked with the correct designation of the contained larvae. Almost any type dish or pan can be used to rear larvae satisfactorily, but enamelware and glass are easiest to keep clean. The live yeast and powdered dog biscuit which are used for food are ground with a mortar and

pestle and a small amount dusted on the surface of the water twice daily. It is important that not too much food be put in at a time. An excessive amount often causes a thick scum to cover the entire surface of the water and kill the larvae, which are not primarily surface feeders. This condition may usually be avoided by feeding a small amount more often, and by agitating the water at the time of feeding. We have seldom found it necessary to change the water in the pans although some is added every few days to keep the depth at about 2 cm. This procedure is necessary in order to prevent drying out which is an ever-present danger in a warm or air-conditioned room. Many pans can be kept in a small space on adjustable shelves.

Pupae.—As the larvae pupate they are collected from the larval pans with the aid of a four-inch wide-mouth pipette with a rubber bulb, and are handled in one of two ways. If records of each individual are not important, the pupae are placed in clean water in a small-sized pan which in turn is placed in an empty cage or regular colony cage as desired. They are allowed to emerge and thus constitute the adults of the next filial generation. As additional larvae pupate, they are daily placed in the same pan, so that the emergence of a generation may require as much as a week. Prunes or raisins are placed in the cage as before, and the cycle repeated. If data on each individual are required, pupae may be isolated in clean water in shell vials, and after the adults emerge they may be liberated into the stock cages.

Handling techniques.—For examination of adults immobilization by either ethyl or methyl ether is satisfactory. The anaesthetization is rapid, recovery fairly slow and regular. If the adults show signs of too rapid revival, they may be re-etherized in a shell vial, or on the microscope stage. In the latter case, it is convenient to use a petri dish with a small square of blotting paper taped to the bottom. A few drops of ether on the paper and the dish inverted over the adults serves as a very satisfactory etherizing chamber. Adults under ether are best handled with camels'-hair brushes or with fine needles.

Wide-mouthed pipettes are used exclusively for handling larvae and pupae. These can be easily and cheaply made from glass tubing or from ordinary pipettes by breaking off the tip and flaming the edges. A standard insect aspirator is used to collect adults from cages when necessary.

Permanent preservation.—Eggs, larvae and pupae may be preserved in 70% alcohol in ½-dram vials. In experiments with larval characteristics, we routinely mount 4th instar skins on microscope slides ten to a slide, mounted in euparal. Adults may be mounted on pins or kept as dried specimens in small cardboard boxes half-filled with crushed cleansing tissue. Routine mounts of male genitalia are made on standard microscope slides.—JAMES B. KITZMILLER AND DON W. MICKS

A Record of the Blind Isopod, Asellus tridentatus, from an Open Stream Habitat

The subterranean isopod, *Asellus tridentatus* (Hungerford), like other colorless and blind species, is found typically in cisterns, wells, caves, and underground streams. Its lack of eyes and pigment are correlated with its habitat of very low light intensity or total darkness. On March 27, 1953, the writer collected 16 specimens (and left about an equal number) from a headwater drainage stream of the Embarrass River in Champaign County, Illinois. They were mixed with an abundant population of another isopod, *Lirceus garmani* Hubricht and Mackin. Both species were found on the lower surface of a saturated piece of corrugated cardboard resting on the bottom of the shallow brook. While this is a normal habitat for *L. garmani*, it is unusual for the cave-dwelling *A. tridentatus*. Dr. J. G. Mackin, Agricultural and Mechanical College of Texas, kindly checked and identified the species.—RALPH W. DEXTER, Kent State University, Kent, Ohio.